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The efficacy and safety of recombinant human TNK tissue-type plasminogen activator (rhTNK-tPA) in comparison with alteplase (rt-PA) as fibrinolytic therapy in patients with acute ST-segment elevation myocardial infarction (China TNK STEMI): Design and rationale for a multicenter, randomized, open-label, parallel-group, active controlled non-inferiority trial

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The efficacy and safety of recombinant human TNK tissue-type plasminogen activator (rhTNK-tPA) in comparison with alteplase (rt-PA) as fibrinolytic therapy in patients with acute ST-segment elevation myocardial infarction (China TNK STEMI): Design and rationale for a multicenter, randomized, open-label, parallel-group, active controlled non-inferiority trial

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Abstract

Introduction: Fibrinolytic therapy continues to play an important role in reperfusion for the patients with acute ST-segment elevation myocardial infarction (STEMI) in settings and occasions where primary percutaneous coronary intervention is not available. Recombinant human TNK tissue-type plasminogen activator (rhTNK-tPA,) has been developed in China. The study is to evaluate the efficacy and safety of rhTNK-tPA in lowering major adverse cardiovascular and cerebrovascular events (MACCEs) in Chinese STEMI patients.

Methods and Analysis: The study is designed as a multicenter, randomized, open-label, parallel-group, active controlled non-inferiority trial with balanced randomization (1:1) in patients with STEMI. The planned sample size is 6,200 participants (or 3,100 per arm). Participants with STEMI are randomized to receive either rhTNK-tPA or rt-PA (alteplase, Actilyse®, Boehringer Ingelheim), with stratification by research center, age and the time from symptom onset to randomization. All patients will receive concomitant antiplatelet and anticoagulant therapy prior to fibrinolytic therapy. The participants assigned to the intervention group will receive an intravenous bolus of 16 mg rhTNK-tPA, while those assigned to the control group will receive an intravenous bolus of 8 mg rt-PA followed by 42 mg infusion over 90 mins. Other medications can also be administered at the discretion of treating physicians. all participants will be followed up for the primary study endpoint, the occurrence of MACCEs within 30 days after fibrinolytic therapy, which will be adjudicated by an independent Clinical Endpoint Committee blinded to treatment assignment. Both intention-to-treat and per-protocol analyses will be done for the primary analyses.

Ethics and Dissemination: The results will be disseminated in peer review journals and academic conferences. This multicenter randomized controlled trial will provide high-quality data about the efficacy and safety of rhTNK-tPA and its application would help improve timely reperfusion therapy and hence the treatment outcomes of STEMI patients.

Trial registration number: NCT02835534.

Article summary

Strengths and limitations of this study

- rhTNK-tPA has been newly developed by Chinese company (Guangzhou Recomgen Biotech Co.,Ltd.).
- The first randomized controlled trial evaluating the efficacy and safety of rhTNK-tPA among STEMI patients in China.
- Over 6,200 participants recruited in about 150 hospitals in China.
- Pharmacoeconomic evaluation and economic analysis involved.

Keywords: Recombinant human TNK tissue-type plasminogen activator, Alteplase, Myocardial infarction, Fibrinolysis, China

Background

The total number of cardiovascular and cerebrovascular deaths is increasing due to the ageing of populations and the changing of lifestyles, accounting for about 30% of all deaths globally in 2013[1] and over 40% in China[2]. ST-segment elevation myocardial infarction (STEMI), characterized by thrombosis and occlusion of coronary arteries resulting from the formation and rupture of coronary atherosclerotic plaques, is a common and serious cardiovascular disorder with high morbidity and mortality[3], and has become a major public health problem in China[4].

Obtaining complete and sustained patency of infarct-related artery (IRA) by restoring coronary flow and reperfusion as early as possible is currently proven as the key to reduce mortality and morbidity of STEMI patients[3 5 6]. For STEMI patients, percutaneous coronary interventions (PCI) improve patient survival earlier and are associated with better outcomes compared with thrombolytic drugs[7-10] However, a large proportion of patients are unable to undergo PCI due to various reasons, especially in settings and occasions where the PCI is not accessible within required time frame or not affordable [11 12]. In addition, there are evidences that within 3 hours of onset fibrinolytic therapy is as effective as PCI therapy[13]. Therefore, fibrinolytic therapy continues to be a preferable choice for those STEMI patients with poor access to health care.

Rapid infusion of the recombinant human tissue-type plasminogen activator (rt-PA, alteplase, Actilyse®, Boehringer Ingelheim), in combination with aspirin and heparin, is the most stable and credible strategy for fibrinolytic therapy among STEMI patients, showing significant effects in reperfusion of IRA, protecting left ventricular function and reducing mortality[14]. However, rt-PA has relatively quick plasma clearance and short half-life, therefore, high dose intravenous infusion is required to maintain effective drug concentration resulting in inconvenience in first-aid. Recombinant human TNK tissue-type plasminogen activator (rhTNK-tPA, Recomlyse®, Guangzhou Recomgen Biotech Co., Ltd.) is a genetically engineered variant of rt-PA designed to be more fibrin-specific[15]. Tenecteplase (TNKase™, Genentech Inc.), also recombinant TNK-tPA, has been approved by the US Food and Drug Administration (FDA) in 2000, and it has significant advantages compared with rt-PA, including ease administration, longer half-life and better fibrin specificity[14]. Assessment of the Safety of a New Thrombolytic (ASSENT-3) trial showed that the incidence rates of

the composite endpoints of 30-day mortality, in-hospital reinfarction, or in-hospital refractory ischaemia were 6.1% for full-dose tenecteplase plus enoxaparin, and 8.8% for full-dose tenecteplase plus unfractionated heparin[16].

rhTNK-tPA, with the same amino acid sequence with tenecteplase, has been developed by Guangzhou Recomgen Biotech Co.,Ltd., China. A multicenter, randomized, active-controlled phase Π trial was conducted to explore the efficacy and safety of rhTNK-tPA (124 cases) and rt-PA (127 cases)[17]. Thrombolysis in MI (TIMI) flow grade 2-3 in the IRA was significantly increased in rhTNK-tPA group compared with rt-PA group (82.8% vs. 67.4%). There was no significant difference in the incidence of adverse events regarding 30-day mortality, 30-day re-infarction, recurrence of myocardial ischemia, target vessel revascularization, heart failure and stroke. To further validate the efficacy and safety of rhTNK-tPA in Chinese STEMI patients, a large sample study would be conducted among STEMI patients in approximately 150 hospitals all over China.

Study design

Trial design and Setting

The study is conducted as a multicenter, randomized, open-label, parallel-group, active controlled non-inferiority trial with balanced randomization (1:1) in patients with STEMI. After giving written informed consent, all eligible participants are randomly assigned to receive fibrinolytic therapy either with rhTNK-tPA or rt-PA. Central randomization is carried out by an interactive Web-based system using the dynamic allocation method stratified by research center, age (≤60 years vs. >60 years) and the time from symptom onset to randomization (≤3 hours vs. 3~6 hours). Participants are recruited continuously by physicians who are responsible for fibrinolytic therapy in approximately 150 hospitals across the country.

Ethical approval

The protocol and informed consent form have been reviewed and approved by the Institutional Review Boards of Fuwai Hospital, Chinese Academy of Medical Sciences in Beijing, China, and the study has been registered at www.clinicaltrials.gov (NCT02835534). Figure 1 illustrates the flow diagram of the study for both the intervention and control groups.

Study population

6,200 participants would be recruited from May, 2016 in about 150 hospitals in China. To be eligible, participants should meet all of following criteria: (1) aged 18–70 years; (2) being diagnosed as acute STEMI, presenting with typical ischemic chest pain lasting for ≥30 mins and ≥0.1 mV ST-segment elevation in ≥2 limb leads or ≥0.2 mV ST-segment elevation in ≥2 contiguous precordial leads; (3) duration from onset of symptoms (typical ischemic chest pain) to randomization ≤6 hours; (4) unable to undergo primary PCI within 1 hr or expected door-to-balloon time >90 mins; (5) willing to participate and provide written informed consent.

Patients meeting any of the following criteria would be excluded: (1) being diagnosed as any of the following conditions: non-ST-segment-elevation acute MI or unstable angina pectoris; reinfarction; cardiogenic shock; suspected aortic dissection; new-onset left bundle branch block diagnosed by electrocardiogram; (2) having any contradiction to fibrinolysis (referring to Chinese Guideline for Diagnosis and Treatment of STEMI , 2015 Edition), including: systolic blood pressure >180 mm Hg or diastolic blood pressure >110 mm Hg or both, and unresponsive to blood-pressure-lowering treatment; history of intracerebral hemorrhage, cryptogenic stroke, or cerebral ischemic stroke within 3 months; abnormality in cerebral vascular structure or intracranial malignant tumors; active hemorrhage, high risk of hemorrhage, or active peptic ulcer disease; severe facial or head trauma within 3 months; major surgery of head and spinal within 2 months; visceral hemorrhage in the previous 4 weeks; major surgery or trauma in the previous 3 weeks; cardiopulmonary resuscitation lasted >10 mins or endotracheal intubation; vascular punctures with hemostasis site unable to be compressed within 2 weeks; current therapy with warfarin, dabigatran, rivaroxaban or glycoprotein IIb/IIIa inhibitors; (3) having other major illnesses that would expose the subject at inordinate risk: indications of cardiac rupture; acute pericarditis, infective endocarditis, acute myocarditis, septic thrombophlebitis or severe infection accompanied with arteriovenous fistula; highly suspected thrombus in left heart chamber, such as mitral stenosis with atrial fibrillation; damage to the central nervous system, such as intracranial tumor, aneurysm, intracranial or spinal canal surgery; severe renal or hepatic dysfunction, or severe hematonosis; maligancy; diabetic hemorrhagic retinopathy or other hemorrhagic ophthalmopathy; history of PCI or coronary artery bypass graft (CABG); fibrinolytic therapy prior to admission; weight less than 50 kg;

falling off or other trauma occurring after the onset of ongoing MI; (4) currently participating in other interventional trial; (5) allergic to rhTNK-tPA and/or rt-PA; (6) pregnancy or lactation; (7) inability or unwilling to follow the protocol due to mental disorder; and (8) any other conditions that the investigator judges make the potential participants unfit for participating in the study.

Participants can withdraw from the study at any time for any reason without any consequences. For every participant who withdraws from the study, the related information collected for early termination and the reasons for withdrawal should be recorded.

Randomization and blinding

Central randomization via interactive web response system (IWRS, Medidata Balance) will be carried out by Peking University Clinical Research Institute, which is independent to the trial administration office. Dynamic randomization will be conducted with varying block size and will be stratified for research center, age (≤60 years vs. >60 years) and the time from symptom onset to randomization (≤3 hours vs. 3~6 hours). The participant is randomized to either intervention group (rhTNK-tPA) or control group (rt-PA) on 1:1 ratio. The allocation sequence is computer-generated and the randomization list is not known to the investigators.

Given the nature of intervention, allocation status cannot be blinded to the participants and investigators due to different methods of administration for intervention and control treatments. However, the primary outcome (major adverse cardiovascular events, MACCEs) and safety indicators will be evaluated by an independent Clinical Endpoint Committee (CEC) which is blinded to treatment assignment. In addition, coronary angiography results will be uniformly reviewed by the core laboratory which is blinded to treatment assignment and TIMI flow grade will be determined accordingly.

Study treatments

All patients will receive concomitant antiplatelet and anticoagulant therapy prior to fibrinolytic therapy according to guideline-based clinical practice. In our study, enoxaparin is the primary drug for anticoagulant co-therapy, however, unfractionated heparin could be used in replace of enoxaparin if enoxaparin is not available. Participants administrated with enoxaparin will receive an intravenous bolus of 30 mg

followed by (after 15 mins) the first subcutaneous dose of 1.0 mg/kg. The subcutaneous dose will be repeated every 12 hours up to a maximum total 8 days. The first two subcutaneous doses could not exceed 100 mg. However, the subcutaneous dose will be repeated every 24 hours for participants whose creatinine clearance rate is <30 ml/min. Participants administrated with unfractionated heparin will receive an intravenous bolus of 4000 U prior to fibrinolytic therapy and initial infusion of 12 U/kg per hour (up to a maximum of 1000U/hour) after fibrinolytic therapy adjusted to maintain an activated partial thromboplastin time of 50-70 s for 24-48 hours with subsequent heparin administration left to the discretion of the investigator. Antiplatelet therapy consists of aspirin in a 300-mg p.o. loading dose prior to fibrinolytic therapy followed by 100 mg daily and clopidogrel in a 300-mg p.o. loading dose prior to fibrinolytic therapy followed by 75 mg daily.

All participants assigned to intervention group will receive an intravenous bolus of 16 mg rhTNK-tPA. The participants assigned to control group will receive an intravenous bolus of 8 mg tPA followed by 42 mg infusion over 90 mins referring to dosing regimen in the TPA/Urokinase Comparisons in China (TUCC) trial[18].

In addition to specified therapies in our study, other medications can also be administered at the discretion of responsible physicians. The decision to proceed further with PCI after the fibrinolytic therapy is left to the investigator's judgement. Rescue PCI will be performed as soon as possible if fibrinolysis fails. Coronary angiography is suggested to be performed 3 to 24 hours after fibrinolytic therapy. The administration dose of unfractionated heparin could be adjusted to reach an activated clotting time of 250-300 sec during implementing PCI in the catheterization laboratory. Additional dosage of anticoagulant therapy is not necessary for rescue PCI if enoxaparin is used as the primary drug for concomitant therapy. However, if PCI is performed within 3 to 24 hours of receiving fibrinolytic therapy, additional dosage of enoxaparin is also not necessary when last subcutaneous dose is given in 8 hours, and an intravenous bolus of 0.3mg/kg enoxaparin will be added when last subcutaneous dose is given in 8 to 12 hours. In hospitals unable to implement coronary revascularization, participants can be transferred to a collaborative tertiary hospital equipped with facilities to perform PCI or coronary angiography when appropriate.

All participants will be closely monitored within 24 hours of fibrinolytic therapy. During the period, 12 lead electrocardiogram (18 lead electrocardiogram for posterior wall and right ventricular MI) examination will be repeated at 30, 60, 90 and 120

mins after fibrinolysis. When appropriate, electrocardiogram examination could be done at the discretion of responsible physicians. Clinical symptoms and signs should be evaluated, especially for duration and relief of chest pain. Creatine kinase-MB (CK-MB) and cardiac troponin (cTn) (if available) will be detected at 10, 12, 14, 16, 18 and 24 hours after symptom onset. TIMI flow grade will be evaluated and recorded if coronary angiography is done within 24 hours of fibrinolytic therapy. In addition, IRA patency will also be evaluated according to non-invasive clinical indexes mentioned above within 24 hours of fibrinolytic therapy.

Baseline assessment and follow-up

The study consists of three phases: baseline assessment, in-hospital follow-up and follow-up after discharge. For each phase the main information collected are described below. Figure 2 shows an overview of the most important data.

Baseline assessment

After receiving written informed consent and checking inclusion/exclusion criteria, responsible cardiologist needs to collect required data using a uniform electronic CRF. Data to be collected at baseline includes demographic characteristics, physical examination, vital signs at hospital admission, history of present illness (onset time of chest pain, admission time and duration of chest pain), cardiac function with Killip class, past medical history (MI, hypertension, diabetes mellitus, hyperlipemia, arrhythmia, peptic ulcer and stroke), past therapeutic history (PCI, CABG, medications for cardiovascular disease), smoking history, laboratory examination (blood routine examination, blood biochemistry, routine urine test and myocardial damage biomarker), 18 lead electrocardiogram and adverse events. After randomization, eligible participants will receive their planned treatment (rhTNK-tPA or rt-PA) and are followed up following the same schedule for both arms (Figure 2). The assigned treatment, medication time of fibrinolytic therapy, antiplatelet and anticoagulant therapy, other concomitant medications and adverse events should be recorded in detail.

In-hospital follow-up

Before the patient's discharge, the responsible investigator needs to collect all data in relevance to identify and diagnose the study outcomes, using a uniform e-CRF. Please refer to the following section of primary and secondary outcomes for details.

30-days follow-up

All randomized participants are planned to be followed-up at 30 (±3) days after fibrinolysis, with both face-to-face interview and telephone follow-up acceptable. Please refer to the following section of primary and secondary outcomes for details.

Study endpoints

The primary study endpoint is the occurrence of MACCEs within 30 days of fibrinolytic therapy, including all causes of death, non-fatal reinfarction, non-fatal stroke (both ischemic and hemorrhagic), PCI due to thrombolysis failure and PCI due to reocclusion. MACCEs will be adjudicated by an independent CEC, the members are not aware of treatment assignment.

Secondary endpoints are a) TIMI flow grade 3 in the IRA within 24 hours of fibrinolytic therapy according to coronary angiography (be restricted to participants with available coronary angiography within 24 hours of fibrinolytic therapy). b) IRA patency within 24 hours of fibrinolytic therapy diagnosed by non-invasive clinical indexes, IRA patency can be determined if any two out of the following 4 items (3+4 excluded) can be achieved: 1) reduction of elevated ST-segment ≥50% by electrocardiogram is achieved within 60-90 mins of receiving fibrinolytic therapy; 2) the time to peak cTn concentration is advanced to ≤12 hours of symptom onset and the time to CK-MB concentration is advanced to ≤14 hours of symptom onset; 3) significant relief of chest pain within 2 hours of fibrinolytic therapy; 4) presence of reperfusion arrhythmia within 2 to 3 hours of fibrinolytic therapy, including accelerated idioventricular rhythm, sudden improvement or disappearance of atrioventricular block or bundle branch block, and transient sinus bradycardia or sino-auricular block with or without hypotension among patients with inferior wall MI. c) The occurrence of MACCEs during hospitalization. d) All-cause mortality during hospitalization and within 30 days of fibrinolytic therapy. e) Cardiovascular mortality during hospitalization and within 30 days of fibrinolytic therapy. f) The occurrence of reinfarction during hospitalization. g) The occurrence of new onset or worsen heart failure during hospitalization. h) The occurrence of cardiac shock during hospitalization. i) The occurrence of coronary revascularization within 30 days of fibrinolytic therapy.

Safety endpoints include a) The occurrence of intracranial hemorrhage during hospitalization. b) The occurrence of gastrointestinal tract major bleeding during

hospitalization. c) Bleeding events by severity during hospitalization. d) The frequency and severity of adverse events. Bleeding events will be evaluated according to TIMI bleeding criteria and adjudicated by CEC. Major bleeding is defined as any intracranial bleeding, or clinically overt signs of a hemorrhage that is associated with a drop in Hb of ≥5 g/dL or a hematocrit decrease of ≥15 points. Minor bleeding is defined as any clinically overt sign of a hemorrhage (including imaging) that is associated with a drop in Hb of 3 to <5 g/dL and a hematocrit decrease of 9 to <15 points. Other bleeding events which do not meet above two criteria are classified as minimal bleeding.

In addition, pharmacoeconomic evaluation will be carried out in our study, including medical direct expense for the first hospitalization which can be obtained directly from hospital records, the number of hospital readmission and the frequency of visiting emergency department due to cardiovascular disease within 30 days of fibrinolytic therapy.

Sample size estimation

Data from our unpublished CPACS-3 study in China showed that the incidence of MACCEs was 17.2% among STEMI patients within 30 days of fibrinolytic therapy. We, therefore, assumed that the incidence of MACCEs would be 17% in the control group in this study and we further set a 3% of absolute rate increase as the non-inferiority margin, corresponding to a non-inferiority relative risk margin of 1.176. With these assumptions, 2,923 participants in each arm of the study are required to provide 80% power with the use of two-sided significance level of 5%. Assuming a 5% loss to follow-up rate, enrolment of 6,200 patients (or 3,100 participants per arm) is projected to yield the necessary number of events.

Statistical consideration

Analyses will be made using SAS statistical software (version 9.4, SAS Institute, Cary, NC, USA) by statisticians in Peking University Clinical Research Institute. Baseline characteristics are reported as frequencies and percentages for qualitative variables and mean ± standard deviation (SD) for quantitative variables. The Student t test or Wilcoxon rank-sum test will be used to compare the difference of quantitative baseline characteristics between the intervention and control group. Comparisons on qualitative variables will be undertaken using the Chi-squared test or Fisher's exact as

required. *P* value <0.05 is considered statistically significant.

Both intention-to-treat (ITT) and per-protocol analyses will be done for the primary analyses as is recommended for non-inferiority studies, but principally with reference to per-protocol analysis. Based on ITT principle, full analysis set (FAS) consisting of all randomized patients is used. The occurrence of MACCEs within 30 days of fibrinolytic therapy is compared with Chi-squared test or Fisher's exact as required, and multivariate logistic regression model is used to adjust for treatment effect on baseline characteristics if there is statistical significant difference between two groups. The secondary endpoints will be analyzed with the chi-square test or Fisher's exact test as appropriate to compare the difference between two groups. If inequality of baseline characteristics is detected, the confounding factors will be defined and multivariate logistic regression model will be used to adjust for covariate effects. Adverse events in the two arms, including bleeding events, will also be compared using the chi-square test or Fisher's exact test as appropriate.

Safety data, especially major bleeding events in the two groups will be reported monthly to the data and safety monitoring committee (DSMB). Through the trial period, DSMB will independently evaluate and review the safety data collected and unexpected events according to the rules and guideline of DSMB. For protecting the safety of participants, DSMB will recommend whether the trial should be continued as planned or not after the analysis of these safety data.

Study status

The first participant was enrolled in July, 11, 2016. As of March 2017, recruitment is ongoing at 31 centers in China with a total of 32 patients randomized. Treatment and follow-up of all participants are planned to continue until December 2020.

Discussion

To our best knowledge, this is the first randomized controlled trial to evaluate the efficacy and safety of rhTNK-tPA in comparison with rt-PA as fibrinolytic therapy in STEMI patients in China. PCI use is considerably hampered by several non-system and system barriers in low and middle income countries like China. Fibrinolytic therapy remains as an important option for those STEMI patients with poor access to health care. The efficacy and safety of tenecteplase (TNKase^{TM}) has been determined

in previous studies in TIMI 10A[19], TIMI 10B[20] and ASSENT-1 study[14]. Therefore, the efficacy and safety of rhTNK-tPA (Recomlyse®) can be assumed to be optional as it has the same amino acid sequence with tenecteplase. Once the efficacy and safety of rhTNK-tPA is confirmed in the study, it will provide additional benefit for STEMI patients in China, especially for those patients with poor access to health care.

rhTNK-tPA is a bioengineered variant of rt-PA developed to avoid some of the limitations of rt-PA. rhTNK-tPA is similar to rt-PA but has triple-combination mutant (amino acid substitutions at 3 sites): adding a glycosylation site to position 103, removing a glycosylation site from site 117 and replacing 4 amino acids, lysine, histidine and two arginines with 4 alanines at the third site[20]. There are several potential advantages for rhTNK-tPA generated by these substitutions, including a longer plasma half-life, increased fibrin specificity and higher resistance to inhibition by plasminogen activator inhibitor 1 compared with rt-PA[15 19 20]. Moreover, its bolus administration of fibrinolysis would facilitate rapid and complete administration, reduce the rate of medication errors, and make more feasible the promising strategy of thrombolysis before admission to hospital[21].

ASSENT-2 trial, in which 16949 participants from more than 1,000 hospitals in 29 countries were randomized, showed that single-bolus tenecteplase and front-loaded altiplasehad equivalent effect on 30-day mortality (6.18% vs. 6.15%)[14]. However, in comparison with altiplase, tenecteplase was associated with fewer major bleeding events (4.66% vs. 5.94%, $P<0.001$) other than intracranial haemorrhage (0.93% vs. 0.94%)[14]. The lower risk of major bleeding event persisted in subgroups with different level of risk. The similar rates of 30-day mortality and intracranial haemorrhage, and the lower risk of non-cerebral bleedings showed that tenecteplase offered a safety benefit over altiplase in the treatment of STEMI patients.

Pharmacoeconomic evaluation will be carried out in our study, including medical direct expense for the first hospitalization, the number of hospital readmission and the frequency of visiting emergency department. Economic analysis will be implemented immediately after the time that clinical data are available. From a public health perspective, the study will serve as an important step to understanding cost-effectiveness of fibrinolytic therapy. The economic analysis results can be important reference data to make budgetary and healthcare resource allocation

decisions, particularly considering the high prevalence and seriousness of STEMI in China. Therefore, the economic analysis for fibrinolytic therapy among STEMI patients, in combination with efficacy and safety data, would be of great value for clinicians and health care providers.

The treatment allocation is not blinded for participants and investigators due to different methods of administration between intervention and control group, which is of course a limitation. However, to reduce observer bias in assessment, the primary outcome (MACCEs) will be evaluated by an independent CEC which is blinded to treatment assignment. Further, all statistical analysis will be done by a statistician at Peking University Clinical Research Institute who is not affiliated with the trial.

Not surprisingly, once the efficacy and safety of rhTNK-tPA is confirmed in the study, its application in China would help improve timely reperfusion and the treatment outcomes of STEMI patients based on its potential advantages including ease of bolus administration, longer plasma half-life, increased fibrin specificity and higher resistance to inhibition by plasminogen activator inhibitor.

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Competing interests

Guangzhou Recomgen Biotech Co., Ltd sponsored the clinical study. HBW, PJ, XYY, CY and YFW were responsible for study design and data analysis at the Peking University Clinical Research Institute, but received no direct payment from Guangzhou Recomgen Biotech Co., Ltd. XSZ, HYX, RLG and SBQ acted as clinical investigators in this clinical study, but received no direct payment from Guangzhou Recomgen Biotech Co., Ltd for their roles in conducting the study. QY is the employee of Guangzhou Recomgen Biotech Co., Ltd.

Authors' contributions

HBW, PJ, XSZ, HYX, QY, YFW and SBQ developed the protocol and grant proposal for this project and wrote the manuscript. XYY, CY, and RLG contributed to the protocol and grant proposal. XYY, HYX and CY assisted with writing and editing of the manuscript. The manuscript was amended based on comments from all authors.

All authors read and approved the final manuscript.

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Abbreviations

MI: myocardial infarction;
STEMI: ST-segment elevation myocardial infarction;
IRA: infarct-related artery;
PCI: percutaneous coronary interventions;
Rt-PA: alteplase;
rhTNK-tPA: recombinant human TNK tissue-type plasminogen activator;
FDA: Food and Drug Administration;
CABG: coronary artery bypass graft;
IWRS: interactive web response system;
MACCEs: major adverse cardiovascular events;
CEC: Clinical Endpoint Committee;
TIMI: Thrombolysis in myocardial infarction;
CK-MB: Creatine kinase-MB;
cTn: cardiac troponin;
CI: confidence interval;
SD: standard deviation;
ITT: intention-to-treat;
FAS: full analysis set;
DSMB: data and safety monitoring committee;
ASSENT: Assessment of the Safety of a New Thrombolytic.

References

1. Mortality GBD, Causes of Death C. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2015;**385**(9963):117-71 doi: 10.1016/S0140-6736(14)61682-2[published Online First: Epub Date]|.
2. Zhou M, Wang H, Zhu J, et al. Cause-specific mortality for 240 causes in China during 1990-2013: a systematic subnational analysis for the Global Burden of Disease Study 2013. Lancet 2016;**387**(10015):251-72 doi: 10.1016/S0140-6736(15)00551-6[published Online First: Epub Date]|.
3. American College of Emergency P, Society for Cardiovascular A, Interventions, et al. 2013 ACCF/AHA guideline for the management of ST-elevation myocardial infarction: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Journal of the American College of Cardiology 2013;**61**(4):e78-140 doi: 10.1016/j.jacc.2012.11.019[published Online First: Epub Date]|.
4. Li J, Li X, Wang Q, et al. ST-segment elevation myocardial infarction in China from 2001 to 2011 (the China PEACE-Retrospective Acute Myocardial Infarction Study): a retrospective analysis of hospital data. Lancet 2015;**385**(9966):441-51 doi: 10.1016/S0140-6736(14)60921-1[published Online First: Epub Date]|.
5. Viikila J, Lilleberg J, Tierala I, et al. Outcome up to one year following different reperfusion strategies in acute ST-segment elevation myocardial infarction: the Helsinki-Uusimaa Hospital District registry of ST-Elevation Acute Myocardial Infarction (HUS-STEMI). European heart journal Acute cardiovascular care 2013;**2**(4):371-8 doi: 10.1177/2048872613501985[published Online First: Epub Date]|.
6. Taylor J. 2012 ESC Guidelines on acute myocardial infarction (STEMI). European heart journal 2012;**33**(20):2501-2 doi: 10.1093/eurheartj/ehs213[published Online First: Epub Date]|.
7. Keeley EC, Boura JA, Grines CL. Primary angioplasty versus intravenous thrombolytic therapy for acute myocardial infarction: a quantitative review of 23 randomised trials. Lancet 2003;**361**(9351):13-20 doi: 10.1016/S0140-6736(03)12113-7[published Online First: Epub Date]|.
8. Boersma E, Primary Coronary Angioplasty vs. Thrombolysis G. Does time matter? A pooled analysis of randomized clinical trials comparing primary percutaneous coronary intervention and in-hospital fibrinolysis in acute myocardial infarction patients. European heart journal 2006;**27**(7):779-88 doi: 10.1093/eurheartj/ehi810[published Online First: Epub Date]|.
9. Rathore SS, Curtis JP, Chen J, et al. Association of door-to-balloon time and mortality in patients admitted to hospital with ST elevation myocardial infarction: national cohort study. Bmj 2009;**338**:b1807 doi: 10.1136/bmj.b1807[published Online First: Epub Date]|.
10. Kushner FG, Hand M, Smith SC, Jr., et al. 2009 focused updates: ACC/AHA guidelines for the management of patients with ST-elevation myocardial infarction (updating the 2004 guideline and 2007 focused update) and ACC/AHA/SCAI guidelines on percutaneous coronary intervention (updating the 2005 guideline and 2007 focused update) a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Journal of the American College of Cardiology 2009;**54**(23):2205-41 doi: 10.1016/j.jacc.2009.10.015[published Online First: Epub Date]|.
11. Bradley EH, Nallamothu BK, Herrin J, et al. National efforts to improve door-to-balloon time results

from the Door-to-Balloon Alliance. Journal of the American College of Cardiology 2009;**54**(25):2423-9 doi: 10.1016/j.jacc.2009.11.003[published Online First: Epub Date]|.

12. Xavier D, Pais P, Devereaux PJ, et al. Treatment and outcomes of acute coronary syndromes in India (CREATE): a prospective analysis of registry data. Lancet 2008;**371**(9622):1435-42 doi: 10.1016/S0140-6736(08)60623-6[published Online First: Epub Date]|.
13. Armstrong PW, Gershlick AH, Goldstein P, et al. Fibrinolysis or primary PCI in ST-segment elevation myocardial infarction. The New England journal of medicine 2013;**368**(15):1379-87 doi: 10.1056/NEJMoa1301092[published Online First: Epub Date]|.
14. Assessment of the S, Efficacy of a New Thrombolytic I, Van De Werf F, et al. Single-bolus tenecteplase compared with front-loaded alteplase in acute myocardial infarction: the ASSENT-2 double-blind randomised trial. Lancet 1999;**354**(9180):716-22
15. Keyt BA, Paoni NF, Refino CJ, et al. A faster-acting and more potent form of tissue plasminogen activator. Proceedings of the National Academy of Sciences of the United States of America 1994;**91**(9):3670-4
16. Assessment of the S, Efficacy of a New Thrombolytic Regimen I. Efficacy and safety of tenecteplase in combination with enoxaparin, abciximab, or unfractionated heparin: the ASSENT-3 randomised trial in acute myocardial infarction. Lancet 2001;**358**(9282):605-13 doi: 10.1016/S0140-6736(01)05775-0[published Online First: Epub Date]|.
17. Zhai M, Chen JL, Qiao SB, et al. Efficacy and safety of recombinant human TNK tissue-typeplasminogen activator in patients with acute myocardial infarction. Chinese Journal of New Drugs 2016;**25**(1):82-86
18. Ross AM, Gao R, Coyne KS, et al. A randomized trial confirming the efficacy of reduced dose recombinant tissue plasminogen activator in a Chinese myocardial infarction population and demonstrating superiority to usual dose urokinase: the TUCC trial. American heart journal 2001;**142**(2):244-7 doi: 10.1067/mhj.2001.116963[published Online First: Epub Date]|.
19. Cannon CP, McCabe CH, Gibson CM, et al. TNK-tissue plasminogen activator in acute myocardial infarction. Results of the Thrombolysis in Myocardial Infarction (TIMI) 10A dose-ranging trial. Circulation 1997;**95**(2):351-6
20. Cannon CP, Gibson CM, McCabe CH, et al. TNK-tissue plasminogen activator compared with front-loaded alteplase in acute myocardial infarction: results of the TIMI 10B trial. Thrombolysis in Myocardial Infarction (TIMI) 10B Investigators. Circulation 1998;**98**(25):2805-14
21. Weaver WD, Cerqueira M, Hallstrom AP, et al. Prehospital-initiated vs hospital-initiated thrombolytic therapy. The Myocardial Infarction Triage and Intervention Trial. Jama 1993;**270**(10):1211-6

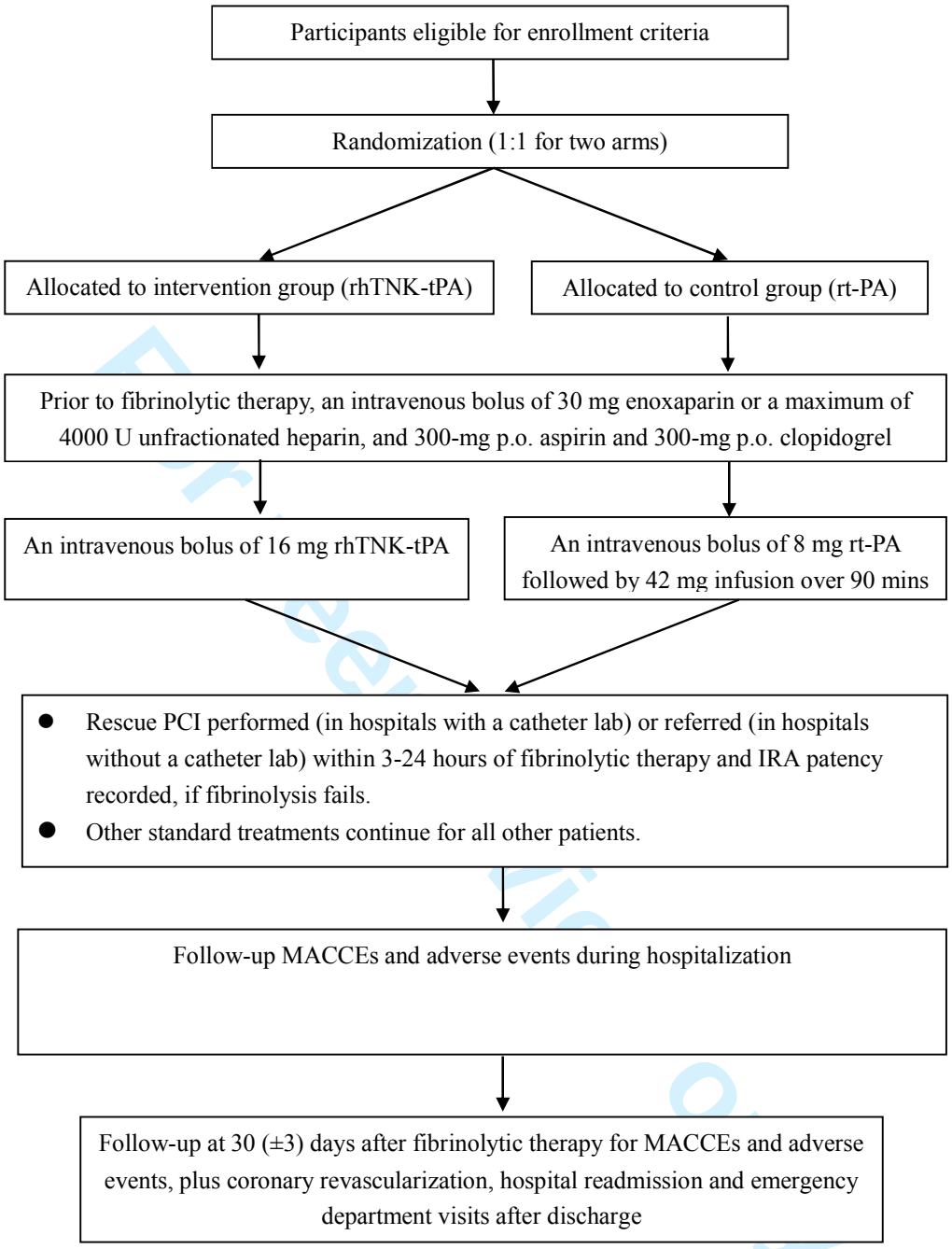

Figure 1. RhTNK-tPA therapeutic efficacy non-inferiority trial flow chart

	Baseline screening	Monitoring after fibrinolysis	In-hospital follow-up	30-days follow-up
Informed consent	●			
Checking enrollment criteria	●			
Demographical characteristics	●			
Medical history[†]	●			
Physical examination	●			
Vital signs	●	●	●	
Clinical symptoms	●	●	●	
Cardiac function[§]	●			
Laboratory examination	●[¶]	●[ζ]	●[ζ]	
Electrocardiogram[Ψ]	●	●	●	
CK-MB[‡]	●	●	●	
Randomization	●			
Drug administration	●			
IRA patency, heart failure and cardiac shock		●	●	
Coronary angiography and ultrasonic cardiogram		●	●	
Coronary revascularization			●	●
Concomitant medication	●	●	●	●
MACCEs[£]		●	●	●
Medical direct expense			●	
Diagnosis and outcome of hospital discharge			●	
Adverse events	●	●	●	●
Health care [ʃ]				●

Figure 2. Baseline screening, assessment, and follow-up schedule.

[†]Including past medical history and past therapeutic history.

[§] Based on Killip class.

[¶] Blood routine examination, blood biochemistry, routine urine test and myocardial damage biomarker.

[ζ] Blood routine examination and myocardial damage biomarker.

[Ψ] 18 lead electrocardiogram prior to fibrinolytic therapy; 12 lead electrocardiogram (18 lead electrocardiogram for posterior wall and right ventricular MI) examination repeated at 30, 60, 90 and 120 mins after fibrinolysis; When appropriate, electrocardiogram examination could be done at the discretion of responsible physicians.

[‡] Detected at 10, 12, 14, 16, 18 and 24 hours after symptom onset, and at second and third day after hospital admission. If available, cTn will be collected at the time points.

[£] Including mortality, non-fatal reinfarction, non-fatal stroke (both ischemic and hemorrhagic stroke), PCI due to thrombolysis failure and PCI due to reocclusion.

[ʃ] Hospital readmission and emergency department visiting due to cardiovascular disease.

BMJ Open

Recombinant human TNK tissue-type plasminogen activator (rhTNK-tPA) versus alteplase (rt-PA) as fibrinolytic therapy for acute ST-segment elevation myocardial infarction (China TNK STEMI): Protocol for a randomized, controlled, non-inferiority trial

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Recombinant human TNK tissue-type plasminogen activator (rhTNK-tPA) versus alteplase (rt-PA) as fibrinolytic therapy for acute ST-segment elevation myocardial infarction (China TNK STEMI): Protocol for a randomized, controlled, non-inferiority trial

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Abstract

Introduction: The study is to evaluate the efficacy and safety of recombinant human TNK tissue-type plasminogen activator (rhTNK-tPA) in lowering major adverse cardiovascular and cerebrovascular events (MACCEs) in Chinese acute ST-segment elevation myocardial infarction (STEMI)patients.

Methods and Analysis: The study is designed as a multicenter, randomized, open-label, non-inferiority trial with balanced randomization (1:1) in patients with STEMI. The planned sample size is 6,200 participants (or 3,100 per arm). Participants with STEMI are randomized to receive either rhTNK-tPA or rt-PA (alteplase, Actilyse®, Boehringer Ingelheim), with stratification by research center, age and the time from symptom onset to randomization. All patients will receive concomitant antiplatelet and anticoagulant therapy prior to fibrinolytic therapy. The participants assigned to the intervention group will receive an intravenous bolus of 16 mg rhTNK-tPA, while those assigned to the control group will receive an intravenous bolus of 8 mg rt-PA followed by 42 mg infusion over 90 mins. Other medications can also be administered at the discretion of cardiologists in charge. All participants will be followed up for the primary study endpoint, the occurrence of MACCEs within 30 days after fibrinolytic therapy, which was defined as a composite end-point comprising all causes of death, non-fatal re-infarction, non-fatal stroke, percutaneous coronary interventions (PCI) due to thrombolysis failure and PCI due to reocclusion. Both intention-to-treat and per-protocol analyses will be done for the primary analyses.

Ethics and Dissemination: The protocol and informed consent form have been reviewed and approved by all participating hospitals. The results will be disseminated in peer review journals and academic conferences. This multicenter randomized controlled trial will provide high-quality data about the efficacy and safety of rhTNK-tPA and once approved its easier use would help improve the application of reperfusion therapy and hence the treatment outcomes of STEMI patients.

Trial registration number: NCT02835534.

Article summary

Strengths and limitations of this study

- rhTNK-tPA has been newly developed by Chinese company (Guangzhou Recomgen Biotech Co.,Ltd.).
- The first randomized controlled trial evaluating the efficacy and safety of rhTNK-tPA among STEMI patients in China.
- Over 6,200 participants recruited in about 150 hospitals in China.
- Pharmacoeconomic evaluation and economic analysis involved.

Keywords: Recombinant human TNK tissue-type plasminogen activator, Alteplase, Myocardial infarction, Fibrinolysis, China

Background

The total number of cardiovascular and cerebrovascular deaths is increasing due to the ageing of populations and the changing of lifestyles, accounting for about 30% of all deaths globally in 2013[1] and over 40% in China[2]. ST-segment elevation myocardial infarction (STEMI), characterized by thrombosis and occlusion of coronary arteries resulting from the formation and rupture of coronary atherosclerotic plaques, is a common and serious cardiovascular disorder with high morbidity and mortality[3], and has become a major public health problem in China[4].

Obtaining complete and sustained patency of infarct-related artery (IRA) by restoring coronary flow and reperfusion as early as possible is currently proven as the key to reduce mortality and morbidity of STEMI patients[3 5 6]. For STEMI patients, percutaneous coronary interventions (PCI) improve patient survival earlier and are associated with better outcomes compared with thrombolytic drugs[7-10] However, a large proportion of patients are unable to undergo PCI due to various reasons, especially in settings and occasions where the PCI is not accessible within required time frame or not affordable [11 12]. In addition, there are evidences that within 3 hours of onset fibrinolytic therapy is as effective as PCI therapy[13]. Therefore, fibrinolytic therapy continues to be a preferable choice for those STEMI patients with poor access to health care.

Rapid infusion of the recombinant human tissue-type plasminogen activator (rt-PA, alteplase, Actilyse®, Boehringer Ingelheim), in combination with aspirin and heparin, is the most stable and credible strategy for fibrinolytic therapy among STEMI patients, showing significant effects in reperfusion of IRA, protecting left ventricular function and reducing mortality[14]. However, rt-PA has relatively quick plasma clearance and short half-life, therefore, high dose intravenous infusion is required to maintain effective drug concentration resulting in inconvenience in first-aid. Recombinant human TNK tissue-type plasminogen activator (rhTNK-tPA, Recomlyse®, Guangzhou Recomgen Biotech Co., Ltd.) is a genetically engineered variant of rt-PA designed to be more fibrin-specific[15]. Tenecteplase (TNKase™, Genentech Inc.), also recombinant TNK-tPA, has been approved by the US Food and Drug Administration (FDA) in year 2000, and it has significant advantages compared with rt-PA, including ease administration, longer half-life and better fibrin specificity[14]. Assessment of the Safety of a New Thrombolytic (ASSENT-3) trial showed that the incidence rates

of the composite endpoints of 30-day mortality, in-hospital reinfarction, or in-hospital refractory ischaemia were 6.1% for full-dose tenecteplase plus enoxaparin, and 8.8% for full-dose tenecteplase plus unfractionated heparin[16].

So far, tPA has been approved by China FDA, but Tenecteplase has not yet entered the Chinese market. rhTNK-tPA, with the same amino acid sequence with tenecteplase, has been developed by Guangzhou Recomgen Biotech Co.,Ltd., China. Previous phase II trial of rhTNK-tPA found that in comparison with rt-PA [17], thrombolysis in MI (TIMI) flow grade 2-3 in the IRA was significantly increased in rhTNK-tPA group compared with rt-PA group (82.8% vs. 67.4%). To further understand the efficacy and safety of rhTNK-tPA in reducing clinical events, a study with large sample is designed to be conducted among STEMI patients in approximately 150 hospitals across China.

Study design

Trial design and Setting

The study is conducted as a multicenter, randomized, open-label, parallel-group, active controlled non-inferiority trial with balanced randomization (1:1) in patients with STEMI. The protocol of the current study was reported adhering to "Standard Protocol Items: Recommendations for Interventional Trials" statement. After giving written informed consent, all eligible participants are randomly assigned to receive fibrinolytic therapy either with rhTNK-tPA or rt-PA. Central randomization is carried out by an interactive Web-based system using the dynamic allocation method stratified by research center, age (≤60 years vs. >60 years) and the time from symptom onset to randomization (≤3 hours vs. 3~6 hours). Participants are recruited continuously by physicians who are responsible for fibrinolytic therapy in approximately 150 hospitals across the country.

Ethical approval

The protocol and informed consent form have been reviewed and approved by all participating hospitals, and the study has been registered at www.clinicaltrials.gov (NCT02835534) on June 12, 2016 and updated on July 13, 2016. Figure 1 illustrates the flow diagram of the study for both the intervention and control groups.

Study population

6,200 participants would be recruited from May, 2016 in about 150 hospitals in China. To be eligible, participants should meet all of following criteria: (1) aged 18–70 years; (2) being diagnosed as acute STEMI, presenting with typical ischemic chest pain lasting for ≥30 mins and ≥0.1 mV ST-segment elevation in ≥2 limb leads or ≥0.2 mV ST-segment elevation in ≥2 contiguous precordial leads; (3) duration from onset of symptoms (typical ischemic chest pain) to randomization ≤6 hours; (4) unable to undergo primary PCI within 1 hr or expected door-to-balloon time >90 mins; (5) willing to participate and provide written informed consent.

Patients meeting any of the following criteria would be excluded: (1) being diagnosed as any of the following conditions: non-ST-segment-elevation acute MI or unstable angina pectoris; reinfarction; cardiogenic shock; suspected aortic dissection; new-onset left bundle branch block diagnosed by electrocardiogram; (2) having any contradiction to fibrinolysis (referring to Chinese Guideline for Diagnosis and Treatment of STEMI , 2015 Edition), including: systolic blood pressure >180 mm Hg or diastolic blood pressure >110 mm Hg or both, and unresponsive to blood-pressure-lowering treatment; history of intracerebral hemorrhage, cryptogenic stroke, or cerebral ischemic stroke within 3 months; abnormality in cerebral vascular structure or intracranial malignant tumors; active hemorrhage, high risk of hemorrhage, or active peptic ulcer disease; severe facial or head trauma within 3 months; major surgery of head and spinal within 2 months; visceral hemorrhage in the previous 4 weeks; major surgery or trauma in the previous 3 weeks; cardiopulmonary resuscitation lasted >10 mins or endotracheal intubation; vascular punctures with hemostasis site unable to be compressed within 2 weeks; current therapy with warfarin, dabigatran, rivaroxaban or glycoprotein IIb/IIIa inhibitors; (3) having other major illnesses that would expose the subject at inordinate risk: indications of cardiac rupture; acute pericarditis, infective endocarditis, acute myocarditis, septic thrombophlebitis or severe infection accompanied with arteriovenous fistula; highly suspected thrombus in left heart chamber, such as mitral stenosis with atrial fibrillation; damage to the central nervous system, such as intracranial tumor, aneurysm, intracranial or spinal canal surgery; severe renal or hepatic dysfunction, or severe hematonosis; maligancy; diabetic hemorrhagic retinopathy or other hemorrhagic ophthalmopathy; history of PCI or coronary artery bypass graft (CABG); fibrinolytic therapy prior to admission; weight less than 50 kg; falling off or other trauma occurring after the onset of ongoing MI; (4) currently

participating in other interventional trial; (5) allergic to rhTNK-tPA and/or rt-PA; (6) pregnancy or lactation; (7) inability or unwilling to follow the protocol due to mental disorder; and (8) any other conditions that the investigator judges make the potential participants unfit for participating in the study.

Participants can withdraw from the study at any time for any reason without any consequences. For every participant who withdraws from the study, the related information collected for early termination and the reasons for withdrawal should be recorded.

Randomization and blinding

Central randomization via interactive web response system (IWRS, Medidata Balance) will be carried out by Peking University Clinical Research Institute, which is independent to the trial administration office. Dynamic randomization will be conducted with varying block size and will be stratified for research center, age (≤60 years vs. >60 years) and the time from symptom onset to randomization (≤3 hours vs. 3~6 hours). The participant is randomized to either intervention group (rhTNK-tPA) or control group (rt-PA) on 1:1 ratio. The allocation sequence is computer-generated and the randomization list is not known to the investigators.

Given the nature of intervention, allocation status cannot be blinded to the participants and investigators due to different methods of administration for intervention and control treatments. However, the primary outcome (major adverse cardiovascular events, MACCEs) and safety indicators will be evaluated by an independent Clinical Endpoint Committee (CEC) which is blinded to treatment assignment. In addition, coronary angiography results will be uniformly reviewed by the core laboratory which is blinded to treatment assignment and TIMI flow grade will be determined accordingly.

Study treatments

All patients will receive concomitant antiplatelet and anticoagulant therapy prior to fibrinolytic therapy according to guideline-based clinical practice. In our study, enoxaparin is the primary drug for anticoagulant co-therapy, however, unfractionated heparin could be used in replace of enoxaparin if enoxaparin is not available. Participants administrated with enoxaparin will receive an intravenous bolus of 30 mg followed by (after 15 mins) the first subcutaneous dose of 1.0 mg/kg. The

subcutaneous dose will be repeated every 12 hours up to a maximum total 8 days. The first two subcutaneous doses could not exceed 100 mg. However, the subcutaneous dose will be repeated every 24 hours for participants whose creatinine clearance rate is <30 ml/min. Participants administrated with unfractionated heparin will receive an intravenous bolus of 4000 U prior to fibrinolytic therapy and initial infusion of 12 U/kg per hour (up to a maximum of 1000U/hour) after fibrinolytic therapy adjusted to maintain an activated partial thromboplastin time of 50-70 s for 24-48 hours with subsequent heparin administration left to the discretion of the investigator. Antiplatelet therapy consists of aspirin in a 300-mg p.o. loading dose prior to fibrinolytic therapy followed by 100 mg daily and clopidogrel in a 300-mg p.o. loading dose prior to fibrinolytic therapy followed by 75 mg daily.

All participants assigned to intervention group will receive an intravenous bolus of 16 mg rhTNK-tPA. The participants assigned to control group will receive an intravenous bolus of 8 mg tPA followed by 42 mg infusion over 90 mins referring to dosing regimen in the TPA/Urokinase Comparisons in China (TUCC) trial[18].

In addition to specified therapies in our study, other medications can also be administered at the discretion of responsible physicians. The decision to proceed further with PCI after the fibrinolytic therapy is left to the investigator's judgement. Rescue PCI will be performed as soon as possible if fibrinolysis fails. Coronary angiography is suggested to be performed 3 to 24 hours after fibrinolytic therapy. The administration dose of unfractionated heparin could be adjusted to reach an activated clotting time of 250-300 sec during implementing PCI in the catheterization laboratory. Additional dosage of anticoagulant therapy is not necessary for rescue PCI if enoxaparin is used as the primary drug for concomitant therapy. However, if PCI is performed within 3 to 24 hours of receiving fibrinolytic therapy, additional dosage of enoxaparin is also not necessary when last subcutaneous dose is given in 8 hours, and an intravenous bolus of 0.3mg/kg enoxaparin will be added when last subcutaneous dose is given in 8 to 12 hours. In hospitals unable to implement coronary revascularization, participants can be transferred to a collaborative tertiary hospital equipped with facilities to perform PCI or coronary angiography when appropriate.

All participants will be closely monitored within 24 hours of fibrinolytic therapy. During the period, 12 lead electrocardiogram (18 lead electrocardiogram for posterior wall and right ventricular MI) examination will be repeated at 30, 60, 90 and 120 mins after fibrinolysis. When appropriate, electrocardiogram examination could be

done at the discretion of responsible physicians. Clinical symptoms and signs should be evaluated, especially for duration and relief of chest pain. Creatine kinase-MB (CK-MB) and cardiac troponin (cTn) (if available) will be detected at 10, 12, 14, 16, 18 and 24 hours after symptom onset. TIMI flow grade will be evaluated and recorded if coronary angiography is done within 24 hours of fibrinolytic therapy. In addition, IRA patency will also be evaluated according to non-invasive clinical indexes mentioned above within 24 hours of fibrinolytic therapy.

Baseline assessment and follow-up

The study consists of three phases: baseline assessment, in-hospital follow-up and follow-up after discharge. For each phase the main information collected are described below. Figure 2 shows an overview of the most important data.

Baseline assessment

After receiving written informed consent and checking inclusion/exclusion criteria, responsible cardiologist needs to collect required data using a uniform electronic CRF. Data to be collected at baseline includes demographic characteristics, physical examination, vital signs at hospital admission, history of present illness (onset time of chest pain, admission time and duration of chest pain), cardiac function with Killip class, past medical history (MI, hypertension, diabetes mellitus, hyperlipemia, arrhythmia, peptic ulcer and stroke), past therapeutic history (PCI, CABG, medications for cardiovascular disease), smoking history, laboratory examination (blood routine examination, blood biochemistry, routine urine test and myocardial damage biomarker), 18 lead electrocardiogram and adverse events. After randomization, eligible participants will receive their planned treatment (rhTNK-tPA or rt-PA) and are followed up following the same schedule for both arms (Figure 2). The assigned treatment, medication time of fibrinolytic therapy, antiplatelet and anticoagulant therapy, other concomitant medications and adverse events should be recorded in detail.

In-hospital follow-up

Before the patient's discharge, the responsible investigator needs to collect all data in relevance to identify and diagnose the study outcomes, using a uniform e-CRF. Please refer to the following section of primary and secondary outcomes for details.

30-days follow-up

All randomized participants are planned to be followed-up at 30 (±3) days after

fibrinolysis, with both face-to-face interview and telephone follow-up acceptable. Please refer to the following section of primary and secondary outcomes for details.

Study endpoints

The primary study endpoint is the occurrence of MACCEs within 30 days of fibrinolytic therapy, including all causes of death, non-fatal reinfarction, non-fatal stroke (both ischemic and hemorrhagic), PCI due to thrombolysis failure and PCI due to reocclusion. MACCEs will be adjudicated by an independent CEC, the members are not aware of treatment assignment.

Secondary endpoints are a) TIMI flow grade 3 in the IRA within 24 hours of fibrinolytic therapy according to coronary angiography (be restricted to participants with available coronary angiography within 24 hours of fibrinolytic therapy). b) IRA patency within 24 hours of fibrinolytic therapy diagnosed by non-invasive clinical indexes [19 20], IRA patency can be determined if any two out of the following 4 items (3+4 excluded) can be achieved: 1) reduction of elevated ST-segment ≥50% by electrocardiogram is achieved within 60-90 mins of receiving fibrinolytic therapy; 2) the time to peak cTn concentration is advanced to ≤12 hours of symptom onset and the time to CK-MB concentration is advanced to ≤14 hours of symptom onset; 3) significant relief of chest pain within 2 hours of fibrinolytic therapy; 4) presence of reperfusion arrhythmia within 2 to 3 hours of fibrinolytic therapy, including accelerated idioventricular rhythm, sudden improvement or disappearance of atrioventricular block or bundle branch block, and transient sinus bradycardia or sino-auricular block with or without hypotension among patients with inferior wall MI. c) The occurrence of MACCEs during hospitalization. d) All-cause mortality during hospitalization and within 30 days of fibrinolytic therapy. e) Cardiovascular mortality during hospitalization and within 30 days of fibrinolytic therapy. f) The occurrence of reinfarction during hospitalization. g) The occurrence of new onset or worsen heart failure during hospitalization. h) The occurrence of cardiac shock during hospitalization. i) The occurrence of coronary revascularization within 30 days of fibrinolytic therapy.

Safety endpoints include a) The occurrence of intracranial hemorrhage during hospitalization. b) The occurrence of gastrointestinal tract major bleeding during hospitalization. c) Bleeding events by severity during hospitalization. d) The

frequency and severity of adverse events. Bleeding events will be evaluated according to TIMI bleeding criteria and adjudicated by CEC. Major bleeding is defined as any intracranial bleeding, or clinically overt signs of a hemorrhage that is associated with a drop in Hb of ≥5 g/dL or a hematocrit decrease of ≥15 points. Minor bleeding is defined as any clinically overt sign of a hemorrhage (including imaging) that is associated with a drop in Hb of 3 to <5 g/dL and a hematocrit decrease of 9 to <15 points. Other bleeding events which do not meet above two criteria are classified as minimal bleeding.

In addition, pharmacoeconomic evaluation will be carried out in our study, including medical direct expense for the first hospitalization which can be obtained directly from hospital records, the number of hospital readmission and the frequency of visiting emergency department due to cardiovascular disease within 30 days of fibrinolytic therapy.

Safety data monitoring

CEC is composed of four cardiovascular specialists and one neurological expert who are recommended and appointed by Peking University Clinical Research Institute. CEC which is blinded to treatment assignment independently evaluates the primary outcome and safety indicators. Additional two secretaries help collect supporting information about endpoints assessment, organize the communication of evaluation committee and summarize the final results.

Safety data, especially major bleeding events in the two groups will be reported to the data and safety monitoring committee (DSMB). As this trial is a non-inferiority study, an interim analysis is not considered and statistical early stopping criteria will not be applied. However, through the trial period, DSMB has full access to the safety data and will independently evaluate and review the safety data collected and unexpected events according to the rules and guideline of DSMB. The safety data analyses should be done after 10%, 25% and 50% patients completing the study procedure respectively, and DSMB may recommend stopping the study anytime based on safety concerns.

Sample size estimation

Data from our unpublished CPACS-3 study in China showed that the incidence of MACCEs was 17.2% among STEMI patients within 30 days of fibrinolytic therapy.

We, therefore, assumed that the incidence of MACCEs would be 17% in the control group in this study and we further set a 3% of absolute rate increase as the non-inferiority margin, corresponding to a non-inferiority relative risk margin of 1.176. With these assumptions, 2,923 participants in each arm of the study are required to provide 80% power with the use of two-sided significance level of 5%. Assuming a 5% loss to follow-up rate, enrolment of 6,200 patients (or 3,100 participants per arm) is projected to yield the necessary number of events.

Statistical consideration

Analyses will be made using SAS statistical software (version 9.4, SAS Institute, Cary, NC, USA) by statisticians in Peking University Clinical Research Institute. Baseline characteristics are reported as frequencies and percentages for qualitative variables and mean ± standard deviation (SD) for quantitative variables. The Student t test or Wilcoxon rank-sum test will be used to compare the difference of quantitative baseline characteristics between the intervention and control group. Comparisons on qualitative variables will be undertaken using the Chi-squared test or Fisher's exact as required. *P* value <0.05 is considered statistically significant.

Both intention-to-treat (ITT) and per-protocol analyses will be done for the primary analyses as is recommended for non-inferiority studies, but principally with reference to per-protocol analysis. Based on ITT principle, full analysis set (FAS) consisting of all randomized patients is used. Survival curves of MACCEs within 30 days of fibrinolytic therapy were estimated by Kaplan-Meier method and compared by log-rank test. The occurrence of MACCEs within 30 days of fibrinolytic therapy is compared with Chi-squared test or Fisher's exact as required, and multivariate logistic regression model is used to adjust for treatment effect on baseline characteristics if there is statistical significant difference between two groups.

The secondary endpoints will be analyzed with the chi-square test or Fisher's exact test as appropriate to compare the difference between two groups. If inequality of baseline characteristics is detected, the confounding factors will be defined and multivariate logistic regression model will be used to adjust for covariate effects. Adverse events in the two arms, including bleeding events, will also be compared using the chi-square test or Fisher's exact test as appropriate.

Study status

The first participant was enrolled in July, 11, 2016. As of March 2017, recruitment is ongoing at 31 centers in China with a total of 32 patients randomized. Treatment and follow-up of all participants are planned to continue until December 2020.

Discussion

To our best knowledge, this is the first randomized controlled trial to evaluate the efficacy and safety of rhTNK-tPA in comparison with rt-PA as fibrinolytic therapy in STEMI patients in China. PCI use is considerably hampered by several non-system and system barriers in low and middle income countries like China. Fibrinolytic therapy remains as an important option for those STEMI patients with poor access to health care. The efficacy and safety of tenecteplase (TNKase™) has been determined in previous studies in TIMI 10A[21], TIMI 10B[22] and ASSENT-1 study[14]. Therefore, the efficacy and safety of rhTNK-tPA (Recomlyse®) can be assumed to be ideal as it has the same amino acid sequence with tenecteplase. Once the efficacy and safety of rhTNK-tPA is confirmed in the study, it will provide additional benefit for STEMI patients in China, especially for those patients with poor access to health care.

rhTNK-tPA is a bioengineered variant of rt-PA developed to avoid some of the limitations of rt-PA. rhTNK-tPA is similar to rt-PA but has triple-combination mutant (amino acid substitutions at 3 sites): adding a glycosylation site to position 103, removing a glycosylation site from site 117 and replacing 4 amino acids, lysine, histidine and two arginines with 4 alanines at the third site[22]. There are several potential advantages for rhTNK-tPA generated by these substitutions, including a longer plasma half-life, increased fibrin specificity and higher resistance to inhibition by plasminogen activator inhibitor 1 compared with rt-PA[15 21 22]. Moreover, its bolus administration of fibrinolysis would facilitate rapid and complete administration, reduce the rate of medication errors, and make more feasible the promising strategy of thrombolysis before admission to hospital[23].

ASSENT-2 trial, in which 16949 participants from more than 1,000 hospitals in 29 countries were randomized, showed that single-bolus tenecteplase and front-loaded altiplase had equivalent effect on 30-day mortality (6.18% vs. 6.15%)[14]. However, in comparison with altiplase, tenecteplase was associated with fewer major bleeding events (4.66% vs. 5.94%, P<0.001) other than intracranial haemorrhage (0.93% vs.

0.94%)[14]. The lower risk of major bleeding event persisted in subgroups with different level of risk. The similar rates of 30-day mortality and intracranial haemorrhage, and the lower risk of non-cerebral bleedings showed that tenecteplase offered a safety benefit over altiplase in the treatment of STEMI patients.

Pharmacoeconomic evaluation will be carried out in our study, including medical direct expense for the first hospitalization, the number of hospital readmission and the frequency of visiting emergency department. Economic analysis will be implemented immediately after the time that clinical data are available. From a public health perspective, the study will serve as an important step to understanding cost-effectiveness of fibrinolytic therapy. The economic analysis results can be important reference data to make budgetary and healthcare resource allocation decisions, particularly considering the high prevalence and seriousness of STEMI in China. Therefore, the economic analysis for fibrinolytic therapy among STEMI patients, in combination with efficacy and safety data, would be of great value for clinicians and health care providers.

The treatment allocation is not blinded for participants and investigators due to different methods of administration between intervention and control group, which is of course a limitation. However, to reduce observer bias in assessment, the primary outcome (MACCEs) will be evaluated by an independent CEC which is blinded to treatment assignment. Further, all statistical analysis will be done by a statistician at Peking University Clinical Research Institute who is not affiliated with the trial.

Not surprisingly, once the efficacy and safety of rhTNK-tPA is confirmed in the study, its application in China would help improve the treatment of STEMI patients based on its potential advantages including ease of bolus administration, longer plasma half-life, increased fibrin specificity and higher resistance to inhibition by plasminogen activator inhibitor.

Funding

This study is supported by Guangzhou Recomgen Biotech Co., Ltd.

Competing interests

Guangzhou Recomgen Biotech Co., Ltd sponsored the clinical study. HBW, PJ, XYY, CY and YFW were responsible for study design and data analysis at the Peking

University Clinical Research Institute, but received no direct payment from Guangzhou Recomgen Biotech Co., Ltd. XSZ, HYX, RLG and SBQ acted as clinical investigators in this clinical study, but received no direct payment from Guangzhou Recomgen Biotech Co., Ltd for their roles in conducting the study. QY is the employee of Guangzhou Recomgen Biotech Co., Ltd. Principal investigator has full access to the final trial data set, but the sponsor doesn't have access to the data.

Authors' contributions

HBW, PJ, XSZ, HYX, QY, YFW and SBQ developed the protocol and grant proposal for this project and wrote the manuscript. XYY, CY, and RLG contributed to the protocol and grant proposal. XYY, HYX and CY assisted with writing and editing of the manuscript. The manuscript was amended based on comments from all authors. All authors read and approved the final manuscript.

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Figure Legend

Figure 1. RhTNK-tPA therapeutic efficacy non-inferiority trial flow chart

Figure 2. Baseline screening, assessment, and follow-up schedule.

†Including past medical history and past therapeutic history.

§ Based on Killip class.

¶ Blood routine examination, blood biochemistry, routine urine test and myocardial damage biomarker.

ζ Blood routine examination and myocardial damage biomarker.

Ψ 18 lead electrocardiogram prior to fibrinolytic therapy; 12 lead electrocardiogram (18 lead electrocardiogram for posterior wall and right ventricular MI) examination repeated at 30, 60, 90 and 120 mins after fibrinolysis; When appropriate, electrocardiogram examination could be done at the discretion of responsible physicians.

‡ Detected at 10, 12, 14, 16, 18 and 24 hours after symptom onset, and at second and third day after hospital admission. If available, cTn will be collected at the time points.

£ Including mortality, non-fatal reinfarction, non-fatal stroke (both ischemic and hemorrhagic

stroke), PCI due to thrombolysis failure and PCI due to reocclusion.

[ʃ] Hospital readmission and emergency department visiting due to cardiovascular disease.

Abbreviations

MI: myocardial infarction;
STEMI: ST-segment elevation myocardial infarction;
IRA: infarct-related artery;
PCI: percutaneous coronary interventions;
Rt-PA: alteplase;
rhTNK-tPA: recombinant human TNK tissue-type plasminogen activator;
FDA: Food and Drug Administration;
CABG: coronary artery bypass graft;
IWRS: interactive web response system;
MACCEs: major adverse cardiovascular events;
CEC: Clinical Endpoint Committee;
TIMI: Thrombolysis in myocardial infarction;
CK-MB: Creatine kinase-MB;
cTn: cardiac troponin;
CI: confidence interval;
SD: standard deviation;
ITT: intention-to-treat;
FAS: full analysis set;
DSMB: data and safety monitoring committee;
ASSENT: Assessment of the Safety of a New Thrombolytic.

References

1. Mortality GBD, Causes of Death C. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2015;**385**(9963):117-71 doi: 10.1016/S0140-6736(14)61682-2[published Online First: Epub Date]|.
2. Zhou M, Wang H, Zhu J, et al. Cause-specific mortality for 240 causes in China during 1990-2013: a systematic subnational analysis for the Global Burden of Disease Study 2013. Lancet 2016;**387**(10015):251-72 doi: 10.1016/S0140-6736(15)00551-6[published Online First: Epub Date]|.
3. American College of Emergency P, Society for Cardiovascular A, Interventions, et al. 2013 ACCF/AHA guideline for the management of ST-elevation myocardial infarction: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Journal of the American College of Cardiology 2013;**61**(4):e78-140 doi: 10.1016/j.jacc.2012.11.019[published Online First: Epub Date]|.
4. Li J, Li X, Wang Q, et al. ST-segment elevation myocardial infarction in China from 2001 to 2011 (the China PEACE-Retrospective Acute Myocardial Infarction Study): a retrospective analysis of hospital data. Lancet 2015;**385**(9966):441-51 doi: 10.1016/S0140-6736(14)60921-1[published Online First: Epub Date]|.
5. Viikila J, Lilleberg J, Tierala I, et al. Outcome up to one year following different reperfusion strategies in acute ST-segment elevation myocardial infarction: the Helsinki-Uusimaa Hospital District registry of ST-Elevation Acute Myocardial Infarction (HUS-STEMI). European heart journal Acute cardiovascular care 2013;**2**(4):371-8 doi: 10.1177/2048872613501985[published Online First: Epub Date]|.
6. Taylor J. 2012 ESC Guidelines on acute myocardial infarction (STEMI). European heart journal 2012;**33**(20):2501-2 doi: 10.1093/eurheartj/ehs213[published Online First: Epub Date]|.
7. Keeley EC, Boura JA, Grines CL. Primary angioplasty versus intravenous thrombolytic therapy for acute myocardial infarction: a quantitative review of 23 randomised trials. Lancet 2003;**361**(9351):13-20 doi: 10.1016/S0140-6736(03)12113-7[published Online First: Epub Date]|.
8. Boersma E, Primary Coronary Angioplasty vs. Thrombolysis G. Does time matter? A pooled analysis of randomized clinical trials comparing primary percutaneous coronary intervention and in-hospital fibrinolysis in acute myocardial infarction patients. European heart journal 2006;**27**(7):779-88 doi: 10.1093/eurheartj/ehi810[published Online First: Epub Date]|.
9. Rathore SS, Curtis JP, Chen J, et al. Association of door-to-balloon time and mortality in patients admitted to hospital with ST elevation myocardial infarction: national cohort study. Bmj 2009;**338**:b1807 doi: 10.1136/bmj.b1807[published Online First: Epub Date]|.
10. Kushner FG, Hand M, Smith SC, Jr., et al. 2009 focused updates: ACC/AHA guidelines for the management of patients with ST-elevation myocardial infarction (updating the 2004 guideline and 2007 focused update) and ACC/AHA/SCAI guidelines on percutaneous coronary intervention (updating the 2005 guideline and 2007 focused update) a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Journal of the American College of Cardiology 2009;**54**(23):2205-41 doi: 10.1016/j.jacc.2009.10.015[published Online First: Epub Date]|.
11. Bradley EH, Nallamothu BK, Herrin J, et al. National efforts to improve door-to-balloon time results

from the Door-to-Balloon Alliance. Journal of the American College of Cardiology 2009;**54**(25):2423-9 doi: 10.1016/j.jacc.2009.11.003[published Online First: Epub Date]|.

12. Xavier D, Pais P, Devereaux PJ, et al. Treatment and outcomes of acute coronary syndromes in India (CREATE): a prospective analysis of registry data. Lancet 2008;**371**(9622):1435-42 doi: 10.1016/S0140-6736(08)60623-6[published Online First: Epub Date]|.
13. Armstrong PW, Gershlick AH, Goldstein P, et al. Fibrinolysis or primary PCI in ST-segment elevation myocardial infarction. The New England journal of medicine 2013;**368**(15):1379-87 doi: 10.1056/NEJMoa1301092[published Online First: Epub Date]|.
14. Assessment of the S, Efficacy of a New Thrombolytic I, Van De Werf F, et al. Single-bolus tenecteplase compared with front-loaded alteplase in acute myocardial infarction: the ASSENT-2 double-blind randomised trial. Lancet 1999;**354**(9180):716-22
15. Keyt BA, Paoni NF, Refino CJ, et al. A faster-acting and more potent form of tissue plasminogen activator. Proceedings of the National Academy of Sciences of the United States of America 1994;**91**(9):3670-4
16. Assessment of the S, Efficacy of a New Thrombolytic Regimen I. Efficacy and safety of tenecteplase in combination with enoxaparin, abciximab, or unfractionated heparin: the ASSENT-3 randomised trial in acute myocardial infarction. Lancet 2001;**358**(9282):605-13 doi: 10.1016/S0140-6736(01)05775-0[published Online First: Epub Date]|.
17. Zhai M, Chen JL, Qiao SB, et al. Efficacy and safety of recombinant human TNK tissue-typeplasminogen activator in patients with acute myocardial infarction. Chinese Journal of New Drugs 2016;**25**(1):82-86
18. Ross AM, Gao R, Coyne KS, et al. A randomized trial confirming the efficacy of reduced dose recombinant tissue plasminogen activator in a Chinese myocardial infarction population and demonstrating superiority to usual dose urokinase: the TUCC trial. American heart journal 2001;**142**(2):244-7 doi: 10.1067/mhj.2001.116963[published Online First: Epub Date]|.
19. Davies CH, Ormerod OJ. Failed coronary thrombolysis. Lancet 1998;**351**(9110):1191-6
20. Chinese Journal of Cardiology Editorial Board. Reference scheme for treatment of fibrinolytic therapy for acute myocardial infarction. Chinese Journal of Cardiology 1996;**24**(5):328-29. [in Chinese]
21. Cannon CP, McCabe CH, Gibson CM, et al. TNK-tissue plasminogen activator in acute myocardial infarction. Results of the Thrombolysis in Myocardial Infarction (TIMI) 10A dose-ranging trial. Circulation 1997;**95**(2):351-6
22. Cannon CP, Gibson CM, McCabe CH, et al. TNK-tissue plasminogen activator compared with front-loaded alteplase in acute myocardial infarction: results of the TIMI 10B trial. Thrombolysis in Myocardial Infarction (TIMI) 10B Investigators. Circulation 1998;**98**(25):2805-14
23. Weaver WD, Cerqueira M, Hallstrom AP, et al. Prehospital-initiated vs hospital-initiated thrombolytic therapy. The Myocardial Infarction Triage and Intervention Trial. Jama 1993;**270**(10):1211-6

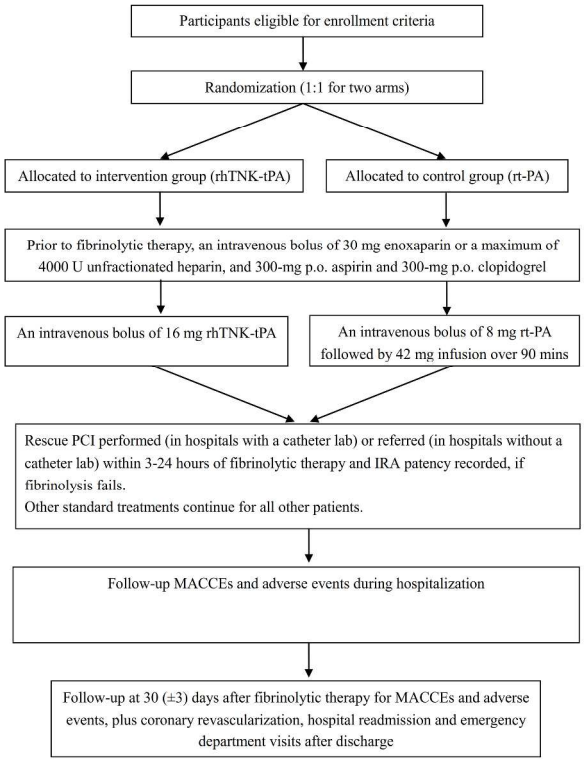

Figure 1

297x420mm (300 x 300 DPI)

	Baseline screening	Monitoring after fibrinolysis	In-hospital follow-up	30-days follow-up
Informed consent	•			
Checking enrollment criteria	•			
Demographical characteristics	•			
Medical history†	•			
Physical examination	•			
Vital signs	•	•	•	
Clinical symptoms	•	•	•	
Cardiac function§	•			
Laboratory examination	•¶	•ξ	•ζ	
Electrocardiogram Ψ	•	•	•	
CK-MB‡	•	•	•	
Randomization	•			
Drug administration	•			
IRA patency, heart failure and cardiac shock		•	•	
Coronary angiography and ultrasonic cardiogram		•	•	
Coronary revascularization			•	•
Concomitant medication	•	•	•	•
MACCEs£		•	•	•
Medical direct expense			•	
Diagnosis and outcome of hospital discharge			•	
Adverse events	•	•	•	•
Health care⊥				•

Figure 2

297x420mm (300 x 300 DPI)

SPIRIT Checking list

Section/Item	Item Number	Description	Checking results	
Administrative information			**Yes /No**	
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	Yes	Recombinant human TNK tissue-type plasminogen activator (rhTNK-tPA) versus alteplase (rt-PA) as fibrinolytic therapy for acute ST-segment elevation myocardial infarction (China TNK STEMI): Protocol for a randomized, controlled, non-inferiority trial
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry.	Yes	The study is registered to Clinical Trials.gov (NCT02835534) with the registry name "The Efficacy and Safety of rhTNK-tPA in Comparison With Alteplase(Rt-PA) as Fibrinolytic Therapy of Acute STEMI".
	2b	All items from the World Health Organization Trial Registration Data Set (**Appendix Table**, available at www.annals.org)	Yes	All items listed in Clinical Trials.gov
Protocol version	3	Date and version identifier	Yes	Version 2.1, 2016-03-03.
Funding	4	Sources and types of financial, material, and other support	Yes	This study is supported by Guangzhou Recomgen Biotech Co., Ltd.
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	Yes	1. Shu-Bin Qiao, Department of Cardiology, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Science and Peking Union Medical College, Principal investigator 2. Hai-Bo Wang, Ping Ji, Xiao-Yan Yan, Chen Yao, Yang-feng Wu,

				Peking University Clinical Research Institute, research design and technical supporting 3. Xing-Shan Zhao, Department of Cardiology, Beijing Jishuitan Hospital, The Fourth Clinical Medical College of Peking University, clinical investigator. 4. Haiyan Xu, Run-Lin Gao. Department of Cardiology, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Science and Peking Union Medical College, clinical investigator. 5. Qin Yang, Guangzhou Recomgen Biotech Co., Ltd., research design.
	5b	Name and contact information for the trial sponsor	Yes	Guangzhou Recomgen Biotech Co., Ltd. Jian-Wen Shi, Tel: 8620-82209996 E-mail: shijianwen@recomgenbio.com
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	Yes	Qin Yang, as the employee of Guangzhou Recomgen Biotech Co., Ltd., has participated in the development of the protocol. But she doesn't engage in data collection, study management, data analysis or data interpretation without decision authority on paper publication.
	5d	Composition, roles, and responsibilities of the coordinating center, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see item 21a for DMC)	Yes	Participants are recruited continuously by physicians who are responsible for fibrinolytic therapy in approximately 150 hospitals across the country. Safety data, especially major bleeding events in the two groups will be reported monthly to the data and safety monitoring committee (DSMB). Through the trial period, DSMB will independently

				evaluate and review the safety data collected and unexpected events according to the rules and guideline of DSMB. For protecting the safety of participants, DSMB will recommend whether the trial should be continued as planned or not after the analysis of these safety data. The primary outcome (major adverse cardiovascular events, MACCEs) and safety indicators will be evaluated by an independent Clinical Endpoint Committee (CEC) which is blinded to treatment assignment. Peking University Clinical Research Institute provides technical supporting for the study, including study design, data management, participants randomization and statistical analysis. Data management department of Peking University Clinical Research Institute is responsible for data quality. Data management related SOPs have been established, including "data management plans" and "data checking plan". Electronic data management system is used in the study and special staff is responsible for data checking. Byrenzhi Co., Ltd. which is independent of the trial administration office is responsible for auditing trial conduct. It is scheduled to audit in the first month after program initiation and one time every three months later.
Introduction				
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining	Yes	Rapid infusion of the recombinant human tissue-type plasminogen activator (rt-PA), in combination with aspirin and heparin, is the most stable and credible strategy for fibrinolytic therapy among

		benefits and harms for each intervention		STEMI patients. However, rt-PA has relatively quick plasma clearance and short half-life, therefore, high dose intravenous infusion is required to maintain effective drug concentration resulting in inconvenience in first-aid. Recombinant human TNK tissue-type plasminogen activator (rhTNK-tPA, Recomlyse®, Guangzhou Recomgen Biotech Co., Ltd.) is a genetically engineered variant of rt-PA, has significant advantages compared with rt-PA, including ease administration, longer half-life and better fibrin specificity.
	6b	Explanation for choice of comparators	Yes	rt-PA has relatively quick plasma clearance and short half-life, therefore, high dose intravenous infusion is required to maintain effective drug concentration resulting in inconvenience in first-aid. Tenecteplase (TNKaseTM, Genentech Inc.), also recombinant TNK-tPA, has been approved by the US Food and Drug Administration (FDA) in 2000, but not entered in Chinese market.
Objectives	7	Specific objectives or hypotheses	Yes	To further validate the efficacy and safety of rhTNK-tPA in Chinese STEMI patients.
Trial design	8	Description of trial design, including type of trial (e.g., parallel group, crossover, factorial, single group), allocation ratio, and framework (e.g., superiority, equivalence, noninferiority, exploratory)	Yes	A multicenter, randomized, open-label, parallel-group, active controlled non-inferiority trial with balanced randomization (1:1).
Methods				
Participants, interventions, and outcomes				
Study setting	9	Description of study settings (e.g., community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study	Yes	The study will be conducted in approximately 150 hospitals where percutaneous coronary intervention is not available across China.

		sites can be obtained		
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centers and individuals who will perform the interventions (e.g., surgeons, psychotherapists)	Yes	Inclusion criteria: (1) aged 18 – 70 years; (2) being diagnosed as acute STEMI, presenting with typical ischemic chest pain lasting for ⩾30 mins and ⩾0.1 mV ST-segment elevation in ⩾2 limb leads or ⩾0.2 mV ST-segment elevation in ⩾2 contiguous precordial leads; (3) duration from onset of symptoms (typical ischemic chest pain) to randomization ⩽6 hours; (4) unable to undergo primary PCI within 1 hr or expected door-to-balloon time >90 mins; (5) willing to participate and provide written informed consent. Exclusion criteria: (1) being diagnosed as any of the following conditions: non-ST-segment-elevation acute MI or unstable angina pectoris; reinfarction; cardiogenic shock; suspected aortic dissection; new-onset left bundle branch block diagnosed by electrocardiogram; (2) having any contradiction to fibrinolysis (referring to Chinese Guideline for Diagnosis and Treatment of STEMI , 2015 Edition), including: systolic blood pressure >180 mm Hg or diastolic blood pressure >110 mm Hg or both, and unresponsive to blood-pressure-lowering treatment; history of intracerebral hemorrhage, cryptogenic stroke, or cerebral ischemic stroke within 3 months; abnormality in cerebral vascular structure or intracranial malignant tumors; active hemorrhage, high risk of hemorrhage, or active peptic ulcer disease; severe facial or head trauma within 3 months; major surgery of head and spinal within 2 months; visceral hemorrhage in the previous 4 weeks; major surgery or trauma in the previous 3 weeks; cardiopulmonary resuscitation lasted >10 mins or endotracheal intubation; vascular punctures with

				hemostasis site unable to be compressed within 2 weeks; current therapy with warfarin, dabigatran, rivaroxaban or glycoprotein IIb/IIIa inhibitors; (3) having other major illnesses that would expose the subject at inordinate risk: indications of cardiac rupture; acute pericarditis, infective endocarditis, acute myocarditis, septic thrombophlebitis or severe infection accompanied with arteriovenous fistula; highly suspected thrombus in left heart chamber, such as mitral stenosis with atrial fibrillation; damage to the central nervous system, such as intracranial tumor, aneurysm, intracranial or spinal canal surgery; severe renal or hepatic dysfunction, or severe hematonosis; maligancy; diabetic hemorrhagic retinopathy or other hemorrhagic ophthalmopathy; history of PCI or coronary artery bypass graft (CABG); fibrinolytic therapy prior to admission; weight less than 50 kg; falling off or other trauma occurring after the onset of ongoing MI; (4) currently participating in other interventional trial; (5) allergic to rhTNK-tPA and/or rt-PA; (6) pregnancy or lactation; (7) inability or unwilling to follow the protocol due to mental disorder; and (8) any other conditions that the investigator judges make the potential participants unfit for participating in the study.
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	Yes	All participants assigned to intervention group will receive an intravenous bolus of 16 mg rhTNK-tPA. The participants assigned to control group will receive an intravenous bolus of 8 mg tPA followed by 42 mg infusion over 90 mins referring to dosing regimen in the TPA/Urokinase Comparisons in China (TUCC) trial.
	11b	Criteria for discontinuing or modifying allocated	Yes	In addition to specified therapies in our study, other medications can

		interventions for a given trial participant (e.g., drug dose change in response to harms, participant request, or improving/worsening disease)		also be administered at the discretion of responsible physicians. The decision to proceed further with PCI after the fibrinolytic therapy is left to the investigator's judgement. Rescue PCI will be performed as soon as possible if fibrinolysis fails. Coronary angiography is suggested to be performed 3 to 24 hours after fibrinolytic therapy. The administration dose of unfractionated heparin could be adjusted to reach an activated clotting time of 250-300 sec during implementing PCI in the catheterization laboratory. Additional dosage of anticoagulant therapy is not necessary for rescue PCI if enoxaparin is used as the primary drug for concomitant therapy. However, if PCI is performed within 3 to 24 hours of receiving fibrinolytic therapy, additional dosage of enoxaparin is also not necessary when last subcutaneous dose is given in 8 hours, and an intravenous bolus of 0.3mg/kg enoxaparin will be added when last subcutaneous dose is given in 8 to 12 hours. In hospitals unable to implement coronary revascularization, participants can be transferred to a collaborative tertiary hospital equipped with facilities to perform PCI or coronary angiography when appropriate.
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (e.g., drug tablet return, laboratory tests)	No	
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Yes	All patients will receive concomitant antiplatelet and anticoagulant therapy prior to fibrinolytic therapy according to guideline-based clinical practice. Enoxaparin is the primary drug for anticoagulant co-therapy, however, unfractionated heparin could be used in replace of enoxaparin if enoxaparin is not available. Antiplatelet therapy

				consists of aspirin in a 300-mg p.o. loading dose prior to fibrinolytic therapy followed by 100 mg daily and clopidogrel in a 300-mg p.o. loading dose prior to fibrinolytic therapy followed by 75 mg daily.
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (e.g., systolic blood pressure), analysis metric (e.g., change from baseline, final value, time to event), method of aggregation (e.g., median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	Yes	The primary study endpoint is the occurrence of MACCEs within 30 days of fibrinolytic therapy, including all causes of death, non-fatal reinfarction, non-fatal stroke (both ischemic and hemorrhagic), PCI due to thrombolysis failure and PCI due to reocclusion. MACCEs will be adjudicated by an independent CEC, the members are not aware of treatment assignment. Secondary endpoints are a) TIMI flow grade 3 in the IRA within 24 hours of fibrinolytic therapy according to coronary angiography (be restricted to participants with available coronary angiography within 24 hours of fibrinolytic therapy). b) IRA patency within 24 hours of fibrinolytic therapy diagnosed by non-invasive clinical indexes, IRA patency can be determined if any two out of the following 4 items (3+4 excluded) can be achieved: 1) reduction of elevated ST-segment ⩾50% by electrocardiogram is achieved within 60-90 mins of receiving fibrinolytic therapy; 2) the time to peak cTn concentration is advanced to ⩽12 hours of symptom onset and the time to CK-MB concentration is advanced to ⩽14 hours of symptom onset; 3) significant relief of chest pain within 2 hours of fibrinolytic therapy; 4) presence of reperfusion arrhythmia within 2 to 3 hours of fibrinolytic therapy, including accelerated idioventricular rhythm, sudden improvement or disappearance of atrioventricular block or bundle branch block, and transient sinus bradycardia or sino-auricular block with or without hypotension

				among patients with inferior wall MI. c) The occurrence of MACCEs during hospitalization. d) All-cause mortality during hospitalization and within 30 days of fibrinolytic therapy. e) Cardiovascular mortality during hospitalization and within 30 days of fibrinolytic therapy. f) The occurrence of reinfarction during hospitalization. g) The occurrence of new onset or worsen heart failure during hospitalization. h) The occurrence of cardiac shock during hospitalization. i) The occurrence of coronary revascularization within 30 days of fibrinolytic therapy.
Participant timeline	13	Time schedule of enrollment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (Figure).	Yes	The study consists of three phases: baseline assessment, in-hospital follow-up and follow-up after discharge. For each phase the main information collected are described below. Figure 2 shows an overview of the most important data.
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	Yes	Data from our unpublished CPACS-3 study in China showed that the incidence of MACCEs was 17.2% among STEMI patients within 30 days of fibrinolytic therapy. We, therefore, assumed that the incidence of MACCEs would be 17% in the control group in this study and we further set a 3% of absolute rate increase as the non-inferiority margin, corresponding to a non-inferiority relative risk margin of 1.176. With these assumptions, 2,923 participants in each arm of the study are required to provide 80% power with the use of two-sided significance level of 5%. Assuming a 5% loss to follow-up rate, enrolment of 6,200 patients (or 3,100 participants per arm) is projected to yield the necessary number of events.
Recruitment	15	Strategies for achieving adequate participant enrollment to reach target sample size	Yes	The physicians of participating hospitals explain the purpose of the study, the procedures and risks/benefits of participation to potential

				subjects or their relatives. After consultation with potential participants and screening their clinical information, the physicians inform patients whether they are eligible for the study.
Assignment of interventions (for controlled trials)				
Allocation Sequence generation	16a	Method of generating the allocation sequence (e.g., computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (e.g., blocking) should be provided in a separate document that is unavailable to those who enroll participants or assign interventions.	Yes	Central randomization via interactive web response system (IWRS, Medidata Balance) will be carried out by Peking University Clinical Research Institute, which is independent to the trial administration office. Dynamic randomization will be conducted with varying block size and will be stratified for research center, age (≤60 years vs. >60 years) and the time from symptom onset to randomization (≤3 hours vs. 3~6 hours). The participant is randomized to either intervention group (rhTNK-tPA) or control group (rt-PA) on 1:1 ratio.
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (e.g., central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	Yes	The allocation sequence is computer-generated and the randomization list is not known to the investigators.
Implementation	16c	Who will generate the allocation sequence, who will enroll participants, and who will assign participants to interventions	Yes	Central randomization is carried out by Peking University Clinical Research Institute, which is independent of the trial administration office. Clinical investigators enroll participants and assign participants to interventions according to randomization.
Blinding (masking)	17a	Who will be blinded after assignment to interventions (e.g., trial participants, care providers, outcome assessors, data analysts), and how	No	Given the nature of intervention, allocation status cannot be blinded to the participants and investigators due to different methods of administration for intervention and control treatments.
	17b	If blinded, circumstances under which unblinding is	No	

		permissible, and procedure for revealing a participant's allocated intervention during the trial		
Data collection, management, and analysis				
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (e.g., duplicate measurements, training of assessors) and a description of study instruments (e.g., questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol.	Yes	Described in the Figure 1 and Figure 2. The study consists of three phases: baseline assessment, in-hospital follow-up and follow-up after discharge. For each phase the main information collected are described below. Figure 2 shows an overview of the most important data. Before the patient's discharge, the responsible investigator needs to collect all data in relevance to identify and diagnose the study outcomes, using a uniform e-CRF. All randomized participants are planned to be followed-up at 30 (±3) days after fibrinolysis, with both face-to-face interview and telephone follow-up acceptable. Please refer to the following section of primary and secondary outcomes for details.
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	Yes	Colleting contact information and providing free treatment to maintain follow-up adherence.
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (e.g., double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol.	Yes	Data management department of Peking University Clinical Research Institute is responsible for data quality. Data management related SOPs have been established, including "data management plans" and "data checking plan". Electronic data management system is used in the study and special staff is responsible for data checking.
Statistical	20a	Statistical methods for analyzing primary and	Yes	Based on ITT principle, full analysis set (FAS) consisting of all

methods		secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol.		randomized patients is used. The occurrence of MACCEs within 30 days of fibrinolytic therapy is compared with Chi-squared test or Fisher's exact as required, and multivariate logistic regression model is used to adjust for treatment effect on baseline characteristics if there is statistical significant difference between two groups. The secondary endpoints will be analyzed with the chi-square test or Fisher's exact test as appropriate to compare the difference between two groups. Adverse events in the two arms, including bleeding events, will also be compared using the chi-square test or Fisher's exact test as appropriate.
	20b	Methods for any additional analyses (e.g., subgroup and adjusted analyses)	Yes	If inequality of baseline characteristics is detected, the confounding factors will be defined and multivariate logistic regression model will be used to adjust for covariate effects.
	20c	Definition of analysis population relating to protocol nonadherence (e.g., as-randomized analysis), and any statistical methods to handle missing data (e.g., multiple imputation)	Yes	Both intention-to-treat (ITT) and per-protocol analyses will be done for the primary analyses as is recommended for non-inferiority studies, but principally with reference to per-protocol analysis.
Monitoring				
Data monitoring	21a	Composition of DMC; summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed.	Yes	The data and safety monitoring committee (DSMB) is composed of two cardiovascular physicians and one neurological expert who are recommended and appointed by Peking University Clinical Research Institute. Safety data, especially major bleeding events in the two groups will be reported monthly to DSMB. Through the trial period, DSMB will independently evaluate and review the safety data collected and unexpected events according to the rules and guideline of DSMB.

				The primary objective of the DSMB is to monitor the safety of the interventions and the validity and integrity of the data from the study. Additionally, the DSMB will evaluate the pace of recruitment and will make recommendations regarding the continuation, modification, or termination of the study. The following will ensure the independence of the DSMB: •Members of the DSMB will not participate as investigators in any study under review and will not be supervised by study investigators. •Members of the DSMB must not have a direct interest in knowing or influencing trial outcome or have a financial or intellectual interest in the outcome of any studies under review. •DSMB members must disclose all pharmaceutical companies, biotechnology companies, and CROs in which they hold financial interest. Members must disclose all consultancies (direct or indirect) with pharmaceutical companies, biotechnology companies, and CROs.
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial		As this trial is a non-inferiority trial, we do not consider an interim analysis appropriate. As an interim analysis will not be performed, statistical early stopping criteria will not be applied. However, DSMB has full access to the safety data and will do a thorough review of these after 10%, 25% and 50% patients completing the study procedure, and may recommend stopping the study anytime based on safety concerns.
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial	Yes	Safety data, especially major bleeding events in the two groups will be reported monthly to the data and safety monitoring committee (DSMB). Through the trial period, DSMB will independently

		interventions or trial conduct		evaluate and review the safety data collected and unexpected events according to the rules and guideline of DSMB. For protecting the safety of participants, DSMB will recommend whether the trial should be continued as planned or not after the analysis of these safety data.
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Yes	Byrenzhi Co., Ltd. which is independent of the trial administration office is responsible for auditing trial conduct. It is scheduled to audit in the first month after program initiation and one time every three months later. The auditing contents include complete status and upgrading of study files, signature of informed consent, source data verification, protocol violation and serious adverse events. A written inspection report will be drafted after audition and the report will be sent to principle investigator and sponsor.
Ethics and dissemination				
Research ethics approval	24	Plans for seeking REC/IRB approval	Yes	The protocol and informed consent form should be reviewed and approved by the Institutional Review Boards of all participating hospitals.
Protocol amendments	25	Plans for communicating important protocol modifications (e.g., changes to eligibility criteria, outcomes, analyses) to relevant parties (e.g., investigators, RECs/IRBs, trial participants, trial registries, journals, regulators)	Yes	Protocol revision should get the approval of both principle investigator and Peking University Clinical Research Institute. In addition, any protocol modifications should be reported to other investigators, RECs/IRBs and trial participants. And the registry information will be revised in trial registries.
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorized surrogates, and how (see item 32)	Yes	Informed consent was done by physicians in the participating hospitals.

	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	No	
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	Yes	The files including personal information will be locked in a cabinet. Only special study staffs, auditor and IRBs have limited access to these files for study purpose. The CRF for collecting research information does not include personal information. And the data published will not include any personal information.
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	Yes	Principal investigators acted as clinical investigators in this clinical study, but received no direct payment from Guangzhou Recomgen Biotech Co., Ltd for their roles in conducting the study.
Access to data	29	Statement of who will have access to the final trial data set, and disclosure of contractual agreements that limit such access for investigators	Yes	Principal investigators have full access to the final trial data set.
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	No	The treatments in intervention and control group are regular in clinical setting without excess harms to participants. The safety advents will be disposed as regular clinical side effects.
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, health care professionals, the public, and other relevant groups (e.g., via publication, reporting in results databases, or other data-sharing arrangements), including any publication restrictions	Yes	The study results will be published in medical journal or communicated with health care professionals in academic conference, but all the personal information of participants is prohibited to disclose.
	31b	Authorship eligibility guidelines and any intended use of professional writers	Yes	Research related investigators are eligible for the authorship.

	31c	Plans, if any, for granting public access to the full protocol, participant-level data set, and statistical code	Yes	Full protocol will be published in medical journal and everybody has public access for it. Only principle investigator has full access to participant-level data set, and statistical code. Other staffs can get the access to study data only if they get grant from principle investigator.
Appendices				
Informed consent materials	32	Model consent form and other related documentation given to participants and authorized surrogates	Yes	Informed consent has been designed, and both participants and investigators should sign inform consent before participating in the study.
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	No	Biological specimens will not be stored for future study.

BMJ Open

Recombinant human TNK tissue-type plasminogen activator (rhTNK-tPA) versus alteplase (rt-PA) as fibrinolytic therapy for acute ST-segment elevation myocardial infarction (China TNK STEMI): Protocol for a randomized, controlled, non-inferiority trial

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Recombinant human TNK tissue-type plasminogen activator (rhTNK-tPA) versus alteplase (rt-PA) as fibrinolytic therapy for acute ST-segment elevation myocardial infarction (China TNK STEMI): Protocol for a randomized, controlled, non-inferiority trial

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Abstract

Introduction: The study is to evaluate the efficacy and safety of recombinant human TNK tissue-type plasminogen activator (rhTNK-tPA) in lowering major adverse cardiovascular and cerebrovascular events (MACCEs) in Chinese acute ST-segment elevation myocardial infarction (STEMI)patients.

Methods and Analysis: The study is designed as a multicenter, randomized, open-label, non-inferiority trial with balanced randomization (1:1) in patients with STEMI. The planned sample size is 6,200 participants (or 3,100 per arm). Participants with STEMI are randomized to receive either rhTNK-tPA or rt-PA (alteplase, Actilyse®, Boehringer Ingelheim), with stratification by research center, age and the time from symptom onset to randomization. All patients will receive concomitant antiplatelet and anticoagulant therapy prior to fibrinolytic therapy. The participants assigned to the intervention group will receive an intravenous bolus of 16 mg rhTNK-tPA, while those assigned to the control group will receive an intravenous bolus of 8 mg rt-PA followed by 42 mg infusion over 90 mins. Other medications can also be administered at the discretion of cardiologists in charge. All participants will be followed up for the primary study endpoint, the occurrence of MACCEs within 30 days after fibrinolytic therapy, which is defined as a composite end-point comprising all causes of death, non-fatal re-infarction, non-fatal stroke, percutaneous coronary interventions (PCI) due to thrombolysis failure and PCI due to reocclusion. Both intention-to-treat and per-protocol analyses will be done for the primary analyses.

Ethics and Dissemination: The protocol and informed consent form have been reviewed and approved by all participating hospitals. The results will be disseminated in peer review journals and academic conferences. This multicenter randomized controlled trial will provide high-quality data about the efficacy and safety of rhTNK-tPA and once approved its easier use would help improve the application of reperfusion therapy and hence the treatment outcomes of STEMI patients.

Trial registration number: NCT02835534.

Article summary

Strengths and limitations of this study

- rhTNK-tPA has been newly developed by Chinese company (Guangzhou

Recomgen Biotech Co.,Ltd.).

- The first randomized controlled trial evaluating the efficacy and safety of rhTNK-tPA among STEMI patients in China.
- Over 6,200 participants recruited in about 150 hospitals in China.
- Pharmacoeconomic evaluation and economic analysis involved.

Keywords: Recombinant human TNK tissue-type plasminogen activator, Alteplase, Myocardial infarction, Fibrinolysis, China

Background

The total number of cardiovascular and cerebrovascular deaths is increasing due to the ageing of populations and the changing of lifestyles, accounting for about 30% of all deaths globally in 2013[1] and over 40% in China[2]. ST-segment elevation myocardial infarction (STEMI), characterized by thrombosis and occlusion of coronary arteries resulting from the formation and rupture of coronary atherosclerotic plaques, is a common and serious cardiovascular disorder with high morbidity and mortality[3], and has become a major public health problem in China[4].

Obtaining complete and sustained patency of infarct-related artery (IRA) by restoring coronary flow and reperfusion as early as possible is currently proven as the key to reduce mortality and morbidity of STEMI patients[3 5 6]. For STEMI patients, percutaneous coronary interventions (PCI) improve patient survival earlier and are associated with better outcomes compared with thrombolytic drugs[7-10] However, a large proportion of patients are unable to undergo PCI due to various reasons, especially in settings and occasions where the PCI is not accessible within required time frame or not affordable [11 12]. In addition, there are evidences that within 3 hours of onset fibrinolytic therapy is as effective as PCI therapy[13]. Therefore, fibrinolytic therapy continues to be a preferable choice for those STEMI patients with poor access to health care.

Rapid infusion of the recombinant human tissue-type plasminogen activator (rt-PA, alteplase, Actilyse®, Boehringer Ingelheim), in combination with aspirin and heparin, is the most stable and credible strategy for fibrinolytic therapy among STEMI patients, showing significant effects in reperfusion of IRA, protecting left ventricular function and reducing mortality[14]. However, rt-PA has relatively quick plasma clearance and short half-life, therefore, high dose intravenous infusion is required to maintain effective drug concentration resulting in inconvenience in first-aid. Recombinant human TNK tissue-type plasminogen activator (rhTNK-tPA, Recomlyse®, Guangzhou Recomgen Biotech Co., Ltd.) is a genetically engineered variant of rt-PA designed to be more fibrin-specific[15]. Tenecteplase (TNKase™, Genentech Inc.), also recombinant TNK-tPA, has been approved by the US Food and Drug Administration (FDA) in year 2000, and it has significant advantages compared with rt-PA, including ease administration, longer half-life and better fibrin specificity[14]. Assessment of the Safety of a New Thrombolytic (ASSENT-3) trial showed that the incidence rates

of the composite endpoints of 30-day mortality, in-hospital reinfarction, or in-hospital refractory ischaemia were 6.1% for full-dose tenecteplase plus enoxaparin, and 8.8% for full-dose tenecteplase plus unfractionated heparin[16].

So far, tPA has been approved by China FDA, but Tenecteplase has not yet entered the Chinese market. rhTNK-tPA, with the same amino acid sequence with tenecteplase, has been developed by Guangzhou Recomgen Biotech Co.,Ltd., China. Previous phase II trial of rhTNK-tPA found that in comparison with rt-PA [17], thrombolysis in MI (TIMI) flow grade 2-3 in the IRA was significantly increased in rhTNK-tPA group compared with rt-PA group (82.8% vs. 67.4%). To further understand the efficacy and safety of rhTNK-tPA in reducing clinical events, a study with large sample is designed to be conducted among STEMI patients in approximately 150 hospitals across China.

Study design

Trial design and Setting

The study is conducted as a multicenter, randomized, open-label, parallel-group, active controlled non-inferiority trial with balanced randomization (1:1) in patients with STEMI. The protocol of the current study was reported adhering to "Standard Protocol Items: Recommendations for Interventional Trials" statement. After giving written informed consent, all eligible participants are randomly assigned to receive fibrinolytic therapy either with rhTNK-tPA or rt-PA. Central randomization is carried out by an interactive Web-based system using the dynamic allocation method stratified by research center, age (≤60 years vs. >60 years) and the time from symptom onset to randomization (≤3 hours vs. 3~6 hours). Participants are recruited continuously by physicians who are responsible for fibrinolytic therapy in approximately 150 hospitals across the country.

Ethical approval

The protocol and informed consent form have been reviewed and approved by all participating hospitals, and the study has been registered at www.clinicaltrials.gov (NCT02835534) on June 12, 2016 and updated on July 13, 2016. However, the registry indicated that the trial was retrospectively registered as the first participant was enrolled in July, 11, 2016. Figure 1 illustrates the flow diagram of the study for both the intervention and control groups.

Study population

6,200 participants would be recruited from May, 2016 in about 150 hospitals in China. To be eligible, participants should meet all of following criteria: (1) aged 18–70 years; (2) being diagnosed as acute STEMI, presenting with typical ischemic chest pain lasting for ≥30 mins and ≥0.1 mV ST-segment elevation in ≥2 limb leads or ≥0.2 mV ST-segment elevation in ≥2 contiguous precordial leads; (3) duration from onset of symptoms (typical ischemic chest pain) to randomization ≤6 hours; (4) unable to undergo primary PCI within 1 hr or expected door-to-balloon time >90 mins; (5) willing to participate and provide written informed consent.

Patients meeting any of the following criteria would be excluded: (1) being diagnosed as any of the following conditions: non-ST-segment-elevation acute MI or unstable angina pectoris; reinfarction; cardiogenic shock; suspected aortic dissection; new-onset left bundle branch block diagnosed by electrocardiogram; (2) having any contradiction to fibrinolysis (referring to Chinese Guideline for Diagnosis and Treatment of STEMI , 2015 Edition), including: systolic blood pressure >180 mm Hg or diastolic blood pressure >110 mm Hg or both, and unresponsive to blood-pressure-lowering treatment; history of intracerebral hemorrhage, cryptogenic stroke, or cerebral ischemic stroke within 3 months; abnormality in cerebral vascular structure or intracranial malignant tumors; active hemorrhage, high risk of hemorrhage, or active peptic ulcer disease; severe facial or head trauma within 3 months; major surgery of head and spinal within 2 months; visceral hemorrhage in the previous 4 weeks; major surgery or trauma in the previous 3 weeks; cardiopulmonary resuscitation lasted >10 mins or endotracheal intubation; vascular punctures with hemostasis site unable to be compressed within 2 weeks; current therapy with warfarin, dabigatran, rivaroxaban or glycoprotein IIb/IIIa inhibitors; (3) having other major illnesses that would expose the subject at inordinate risk: indications of cardiac rupture; acute pericarditis, infective endocarditis, acute myocarditis, septic thrombophlebitis or severe infection accompanied with arteriovenous fistula; highly suspected thrombus in left heart chamber, such as mitral stenosis with atrial fibrillation; damage to the central nervous system, such as intracranial tumor, aneurysm, intracranial or spinal canal surgery; severe renal or hepatic dysfunction, or severe hematonosis; maligancy; diabetic hemorrhagic retinopathy or other hemorrhagic ophthalmopathy; history of PCI or coronary artery

bypass graft (CABG); fibrinolytic therapy prior to admission; weight less than 50 kg; falling off or other trauma occurring after the onset of ongoing MI; (4) currently participating in other interventional trial; (5) allergic to rhTNK-tPA and/or rt-PA; (6) pregnancy or lactation; (7) inability or unwilling to follow the protocol due to mental disorder; and (8) any other conditions that the investigator judges make the potential participants unfit for participating in the study.

Participants can withdraw from the study at any time for any reason without any consequences. For every participant who withdraws from the study, the related information collected for early termination and the reasons for withdrawal should be recorded.

Randomization and blinding

Central randomization via interactive web response system (IWRS, Medidata Balance) will be carried out by Peking University Clinical Research Institute, which is independent to the trial administration office. Dynamic randomization will be conducted with varying block size and will be stratified for research center, age (≤60 years vs. >60 years) and the time from symptom onset to randomization (≤3 hours vs. 3~6 hours). The participant is randomized to either intervention group (rhTNK-tPA) or control group (rt-PA) on 1:1 ratio. The allocation sequence is computer-generated and the randomization list is not known to the investigators.

Given the nature of intervention, allocation status cannot be blinded to the participants and investigators due to different methods of administration for intervention and control treatments. However, the primary outcome (major adverse cardiovascular events, MACCEs) and safety indicators will be evaluated by an independent Clinical Endpoint Committee (CEC) which is blinded to treatment assignment. In addition, coronary angiography results will be uniformly reviewed by the core laboratory which is blinded to treatment assignment and TIMI flow grade will be determined accordingly.

Study treatments

All patients will receive concomitant antiplatelet and anticoagulant therapy prior to fibrinolytic therapy according to guideline-based clinical practice. In our study, enoxaparin is the primary drug for anticoagulant co-therapy, however, unfractionated heparin could be used in replace of enoxaparin if enoxaparin is not available.

Participants administrated with enoxaparin will receive an intravenous bolus of 30 mg followed by (after 15 mins) the first subcutaneous dose of 1.0 mg/kg. The subcutaneous dose will be repeated every 12 hours up to a maximum total 8 days. The first two subcutaneous doses could not exceed 100 mg. However, the subcutaneous dose will be repeated every 24 hours for participants whose creatinine clearance rate is <30 ml/min. Participants administrated with unfractionated heparin will receive an intravenous bolus of 4000 U prior to fibrinolytic therapy and initial infusion of 12 U/kg per hour (up to a maximum of 1000U/hour) after fibrinolytic therapy adjusted to maintain an activated partial thromboplastin time of 50-70 s for 24-48 hours with subsequent heparin administration left to the discretion of the investigator. Antiplatelet therapy consists of aspirin in a 300-mg p.o. loading dose prior to fibrinolytic therapy followed by 100 mg daily and clopidogrel in a 300-mg p.o. loading dose prior to fibrinolytic therapy followed by 75 mg daily.

All participants assigned to intervention group will receive an intravenous bolus of 16 mg rhTNK-tPA. The participants assigned to control group will receive an intravenous bolus of 8 mg tPA followed by 42 mg infusion over 90 mins referring to dosing regimen in the TPA/Urokinase Comparisons in China (TUCC) trial[18].

In addition to specified therapies in our study, other medications can also be administered at the discretion of responsible physicians. The decision to proceed further with PCI after the fibrinolytic therapy is left to the investigator's judgement. Rescue PCI will be performed as soon as possible if fibrinolysis fails. Coronary angiography is suggested to be performed 3 to 24 hours after fibrinolytic therapy. The administration dose of unfractionated heparin could be adjusted to reach an activated clotting time of 250-300 sec during implementing PCI in the catheterization laboratory. Additional dosage of anticoagulant therapy is not necessary for rescue PCI if enoxaparin is used as the primary drug for concomitant therapy. However, if PCI is performed within 3 to 24 hours of receiving fibrinolytic therapy, additional dosage of enoxaparin is also not necessary when last subcutaneous dose is given in 8 hours, and an intravenous bolus of 0.3mg/kg enoxaparin will be added when last subcutaneous dose is given in 8 to 12 hours. In hospitals unable to implement coronary revascularization, participants can be transferred to a collaborative tertiary hospital equipped with facilities to perform PCI or coronary angiography when appropriate.

All participants will be closely monitored within 24 hours of fibrinolytic therapy. During the period, 12 lead electrocardiogram (18 lead electrocardiogram for posterior

wall and right ventricular MI) examination will be repeated at 30, 60, 90 and 120 mins after fibrinolysis. When appropriate, electrocardiogram examination could be done at the discretion of responsible physicians. Clinical symptoms and signs should be evaluated, especially for duration and relief of chest pain. Creatine kinase-MB (CK-MB) and cardiac troponin (cTn) (if available) will be detected at 10, 12, 14, 16, 18 and 24 hours after symptom onset. TIMI flow grade will be evaluated and recorded if coronary angiography is done within 24 hours of fibrinolytic therapy. In addition, IRA patency will also be evaluated according to non-invasive clinical indexes mentioned above within 24 hours of fibrinolytic therapy.

Baseline assessment and follow-up

The study consists of three phases: baseline assessment, in-hospital follow-up and follow-up after discharge. For each phase the main information collected are described below. Figure 2 shows an overview of the most important data.

Baseline assessment

After receiving written informed consent and checking inclusion/exclusion criteria, responsible cardiologist needs to collect required data using a uniform electronic CRF. Data to be collected at baseline includes demographic characteristics, physical examination, vital signs at hospital admission, history of present illness (onset time of chest pain, admission time and duration of chest pain), cardiac function with Killip class, past medical history (MI, hypertension, diabetes mellitus, hyperlipemia, arrhythmia, peptic ulcer and stroke), past therapeutic history (PCI, CABG, medications for cardiovascular disease), smoking history, laboratory examination (blood routine examination, blood biochemistry, routine urine test and myocardial damage biomarker), 18 lead electrocardiogram and adverse events. After randomization, eligible participants will receive their planned treatment (rhTNK-tPA or rt-PA) and are followed up following the same schedule for both arms (Figure 2). The assigned treatment, medication time of fibrinolytic therapy, antiplatelet and anticoagulant therapy, other concomitant medications and adverse events should be recorded in detail.

In-hospital follow-up

Before the patient's discharge, the responsible investigator needs to collect all data in relevance to identify and diagnose the study outcomes, using a uniform e-CRF. Please refer to the following section of primary and secondary outcomes for details.

30-days follow-up

All randomized participants are planned to be followed-up at 30 (±3) days after fibrinolysis, with both face-to-face interview and telephone follow-up acceptable. Please refer to the following section of primary and secondary outcomes for details.

Study endpoints

The primary study endpoint is the occurrence of MACCEs within 30 days of fibrinolytic therapy, including all causes of death, non-fatal reinfarction, non-fatal stroke (both ischemic and hemorrhagic), PCI due to thrombolysis failure and PCI due to reocclusion. MACCEs will be adjudicated by an independent CEC, the members are not aware of treatment assignment.

Secondary endpoints are a) TIMI flow grade 3 in the IRA within 24 hours of fibrinolytic therapy according to coronary angiography (be restricted to participants with available coronary angiography within 24 hours of fibrinolytic therapy). b) IRA patency within 24 hours of fibrinolytic therapy diagnosed by non-invasive clinical indexes [19 20], IRA patency can be determined if any two out of the following 4 items (3+4 excluded) can be achieved: 1) reduction of elevated ST-segment ≥50% by electrocardiogram is achieved within 60-90 mins of receiving fibrinolytic therapy; 2) the time to peak cTn concentration is advanced to ≤12 hours of symptom onset and the time to CK-MB concentration is advanced to ≤14 hours of symptom onset; 3) significant relief of chest pain within 2 hours of fibrinolytic therapy; 4) presence of reperfusion arrhythmia within 2 to 3 hours of fibrinolytic therapy, including accelerated idioventricular rhythm, sudden improvement or disappearance of atrioventricular block or bundle branch block, and transient sinus bradycardia or sino-auricular block with or without hypotension among patients with inferior wall MI. c) The occurrence of MACCEs during hospitalization. d) All-cause mortality during hospitalization and within 30 days of fibrinolytic therapy. e) Cardiovascular mortality during hospitalization and within 30 days of fibrinolytic therapy. f) The occurrence of reinfarction during hospitalization. g) The occurrence of new onset or worsen heart failure during hospitalization. h) The occurrence of cardiac shock during hospitalization. i) The occurrence of coronary revascularization within 30 days of fibrinolytic therapy.

Safety endpoints include a) The occurrence of intracranial hemorrhage during

hospitalization. b) The occurrence of gastrointestinal tract major bleeding during hospitalization. c) Bleeding events by severity during hospitalization. d) The frequency and severity of adverse events. Bleeding events will be evaluated according to TIMI bleeding criteria and adjudicated by CEC. Major bleeding is defined as any intracranial bleeding, or clinically overt signs of a hemorrhage that is associated with a drop in Hb of ≥5 g/dL or a hematocrit decrease of ≥15 points. Minor bleeding is defined as any clinically overt sign of a hemorrhage (including imaging) that is associated with a drop in Hb of 3 to <5 g/dL and a hematocrit decrease of 9 to <15 points. Other bleeding events which do not meet above two criteria are classified as minimal bleeding.

In addition, pharmacoeconomic evaluation will be carried out in our study, including medical direct expense for the first hospitalization which can be obtained directly from hospital records, the number of hospital readmission and the frequency of visiting emergency department due to cardiovascular disease within 30 days of fibrinolytic therapy.

Safety data monitoring

CEC is composed of four cardiovascular specialists and one neurological expert who are recommended and appointed by Peking University Clinical Research Institute. CEC which is blinded to treatment assignment independently evaluates the primary outcome and safety indicators. Additional two secretaries help collect supporting information about endpoints assessment, organize the communication of evaluation committee and summarize the final results.

Safety data, especially major bleeding events in the two groups will be reported to the data and safety monitoring committee (DSMB). As this trial is a non-inferiority study, an interim analysis is not considered and statistical early stopping criteria will not be applied. However, through the trial period, DSMB has full access to the safety data and will independently evaluate and review the safety data collected and unexpected events according to the rules and guideline of DSMB. The safety data analyses should be done after 10%, 25% and 50% patients completing the study procedure respectively, and DSMB may recommend stopping the study anytime based on safety concerns.

Sample size estimation

Data from our unpublished CPACS-3 study in China showed that the incidence of MACCEs was 17.2% among STEMI patients within 30 days of fibrinolytic therapy. We, therefore, assumed that the incidence of MACCEs would be 17% in the control group in this study and we further set a 3% of absolute rate increase as the non-inferiority margin, corresponding to a non-inferiority relative risk margin of 1.176. With these assumptions, 2,923 participants in each arm of the study are required to provide 80% power with the use of two-sided significance level of 5%. Assuming a 5% loss to follow-up rate, enrolment of 6,200 patients (or 3,100 participants per arm) is projected to yield the necessary number of events.

Statistical consideration

Analyses will be made using SAS statistical software (version 9.4, SAS Institute, Cary, NC, USA) by statisticians in Peking University Clinical Research Institute. Baseline characteristics are reported as frequencies and percentages for qualitative variables and mean ± standard deviation (SD) for quantitative variables. The Student t test or Wilcoxon rank-sum test will be used to compare the difference of quantitative baseline characteristics between the intervention and control group. Comparisons on qualitative variables will be undertaken using the Chi-squared test or Fisher's exact as required. *P* value <0.05 is considered statistically significant.

Both intention-to-treat (ITT) and per-protocol analyses will be done for the primary analyses as is recommended for non-inferiority studies, but principally with reference to per-protocol analysis. Based on ITT principle, full analysis set (FAS) consisting of all randomized patients is used. Survival curves of MACCEs within 30 days of fibrinolytic therapy will be estimated by Kaplan-Meier method and compared by log-rank test. The occurrence of MACCEs within 30 days of fibrinolytic therapy is compared with Chi-squared test or Fisher's exact as required, and multivariate logistic regression model is used to adjust for treatment effect on baseline characteristics if there is statistical significant difference between two groups.

The secondary endpoints will be analyzed with the chi-square test or Fisher's exact test as appropriate to compare the difference between two groups. If inequality of baseline characteristics is detected, the confounding factors will be defined and multivariate logistic regression model will be used to adjust for covariate effects. Adverse events in the two arms, including bleeding events, will also be compared using the chi-square test or Fisher's exact test as appropriate.

Study status

The first participant was enrolled in July, 11, 2016. As of March 2017, recruitment is ongoing at 31 centers in China with a total of 32 patients randomized. Treatment and follow-up of all participants are planned to continue until December 2020.

Discussion

To our best knowledge, this is the first randomized controlled trial to evaluate the efficacy and safety of rhTNK-tPA in comparison with rt-PA as fibrinolytic therapy in STEMI patients in China. PCI use is considerably hampered by several non-system and system barriers in low and middle income countries like China. Fibrinolytic therapy remains as an important option for those STEMI patients with poor access to health care. The efficacy and safety of tenecteplase (TNKase™) has been determined in previous studies in TIMI 10A[21], TIMI 10B[22] and ASSENT-1 study[14]. Therefore, the efficacy and safety of rhTNK-tPA (Recomlyse®) can be assumed to be ideal as it has the same amino acid sequence with tenecteplase. Once the efficacy and safety of rhTNK-tPA is confirmed in the study, it will provide additional benefit for STEMI patients in China, especially for those patients with poor access to health care.

rhTNK-tPA is a bioengineered variant of rt-PA developed to avoid some of the limitations of rt-PA. rhTNK-tPA is similar to rt-PA but has triple-combination mutant (amino acid substitutions at 3 sites): adding a glycosylation site to position 103, removing a glycosylation site from site 117 and replacing 4 amino acids, lysine, histidine and two arginines with 4 alanines at the third site[22]. There are several potential advantages for rhTNK-tPA generated by these substitutions, including a longer plasma half-life, increased fibrin specificity and higher resistance to inhibition by plasminogen activator inhibitor 1 compared with rt-PA[15 21 22]. Moreover, its bolus administration of fibrinolysis would facilitate rapid and complete administration, reduce the rate of medication errors, and make more feasible the promising strategy of thrombolysis before admission to hospital[23].

ASSENT-2 trial, in which 16949 participants from more than 1,000 hospitals in 29 countries were randomized, showed that single-bolus tenecteplase and front-loaded altiplase had equivalent effect on 30-day mortality (6.18% vs. 6.15%)[14]. However,

in comparison with altiplase, tenecteplase was associated with fewer major bleeding events (4.66% vs. 5.94%, $P<0.001$) other than intracranial haemorrhage (0.93% vs. 0.94%)[14]. The lower risk of major bleeding event persisted in subgroups with different level of risk. The similar rates of 30-day mortality and intracranial haemorrhage, and the lower risk of non-cerebral bleedings showed that tenecteplase offered a safety benefit over altiplase in the treatment of STEMI patients.

Pharmacoeconomic evaluation will be carried out in our study, including medical direct expense for the first hospitalization, the number of hospital readmission and the frequency of visiting emergency department. Economic analysis will be implemented immediately after the time that clinical data are available. From a public health perspective, the study will serve as an important step to understanding cost-effectiveness of fibrinolytic therapy. The economic analysis results can be important reference data to make budgetary and healthcare resource allocation decisions, particularly considering the high prevalence and seriousness of STEMI in China. Therefore, the economic analysis for fibrinolytic therapy among STEMI patients, in combination with efficacy and safety data, would be of great value for clinicians and health care providers.

The treatment allocation is not blinded for participants and investigators due to different methods of administration between intervention and control group, which is of course a limitation. However, to reduce observer bias in assessment, the primary outcome (MACCEs) will be evaluated by an independent CEC which is blinded to treatment assignment. Further, all statistical analysis will be done by a statistician at Peking University Clinical Research Institute who is not affiliated with the trial.

Not surprisingly, once the efficacy and safety of rhTNK-tPA is confirmed in the study, its application in China would help improve the treatment of STEMI patients based on its potential advantages including ease of bolus administration, longer plasma half-life, increased fibrin specificity and higher resistance to inhibition by plasminogen activator inhibitor.

Funding

This study is supported by Guangzhou Recomgen Biotech Co., Ltd.

Competing interests

Guangzhou Recomgen Biotech Co., Ltd sponsored the clinical study. HBW, PJ, XYY, CY and YFW were responsible for study design and data analysis at the Peking University Clinical Research Institute, but received no direct payment from Guangzhou Recomgen Biotech Co., Ltd. XSZ, HYX, RLG and SBQ acted as clinical investigators in this clinical study, but received no direct payment from Guangzhou Recomgen Biotech Co., Ltd for their roles in conducting the study. QY is the employee of Guangzhou Recomgen Biotech Co., Ltd. Principal investigator has full access to the final trial data set, but the sponsor doesn't have access to the data.

Authors' contributions

HBW, PJ, XSZ, HYX, QY, YFW and SBQ developed the protocol and grant proposal for this project and wrote the manuscript. XYY, CY, and RLG contributed to the protocol and grant proposal. XYY, HYX and CY assisted with writing and editing of the manuscript. The manuscript was amended based on comments from all authors. All authors read and approved the final manuscript.

Acknowledgements

The authors would like to acknowledge all clinical investigators for their great effort to the study conduct and all study participants.

Figure Legend

Figure 1. RhTNK-tPA therapeutic efficacy non-inferiority trial flow chart

Figure 2. Baseline screening, assessment, and follow-up schedule.

[†]Including past medical history and past therapeutic history.

[§] Based on Killip class.

[¶] Blood routine examination, blood biochemistry, routine urine test and myocardial damage biomarker.

[ζ]Blood routine examination and myocardial damage biomarker.

[Ψ] 18 lead electrocardiogram prior to fibrinolytic therapy; 12 lead electrocardiogram (18 lead electrocardiogram for posterior wall and right ventricular MI) examination repeated at 30, 60, 90 and 120 mins after fibrinolysis; When appropriate, electrocardiogram examination could be done at the discretion of responsible physicians.

[‡] Detected at 10, 12, 14, 16, 18 and 24 hours after symptom onset, and at second and third day

after hospital admission. If available, cTn will be collected at the time points.

[£] Including mortality, non-fatal reinfarction, non-fatal stroke (both ischemic and hemorrhagic stroke), PCI due to thrombolysis failure and PCI due to reocclusion.

[ʃ] Hospital readmission and emergency department visiting due to cardiovascular disease.

Abbreviations

MI: myocardial infarction;
STEMI: ST-segment elevation myocardial infarction;
IRA: infarct-related artery;
PCI: percutaneous coronary interventions;
Rt-PA: alteplase;
rhTNK-tPA: recombinant human TNK tissue-type plasminogen activator;
FDA: Food and Drug Administration;
CABG: coronary artery bypass graft;
IWRS: interactive web response system;
MACCEs: major adverse cardiovascular events;
CEC: Clinical Endpoint Committee;
TIMI: Thrombolysis in myocardial infarction;
CK-MB: Creatine kinase-MB;
cTn: cardiac troponin;
CI: confidence interval;
SD: standard deviation;
ITT: intention-to-treat;
FAS: full analysis set;
DSMB: data and safety monitoring committee;
ASSENT: Assessment of the Safety of a New Thrombolytic.

References

1. Mortality GBD, Causes of Death C. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2015;**385**(9963):117-71 doi: 10.1016/S0140-6736(14)61682-2[published Online First: Epub Date]|.
2. Zhou M, Wang H, Zhu J, et al. Cause-specific mortality for 240 causes in China during 1990-2013: a systematic subnational analysis for the Global Burden of Disease Study 2013. Lancet 2016;**387**(10015):251-72 doi: 10.1016/S0140-6736(15)00551-6[published Online First: Epub Date]|.
3. American College of Emergency P, Society for Cardiovascular A, Interventions, et al. 2013 ACCF/AHA guideline for the management of ST-elevation myocardial infarction: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Journal of the American College of Cardiology 2013;**61**(4):e78-140 doi: 10.1016/j.jacc.2012.11.019[published Online First: Epub Date]|.
4. Li J, Li X, Wang Q, et al. ST-segment elevation myocardial infarction in China from 2001 to 2011 (the China PEACE-Retrospective Acute Myocardial Infarction Study): a retrospective analysis of hospital data. Lancet 2015;**385**(9966):441-51 doi: 10.1016/S0140-6736(14)60921-1[published Online First: Epub Date]|.
5. Viikila J, Lilleberg J, Tierala I, et al. Outcome up to one year following different reperfusion strategies in acute ST-segment elevation myocardial infarction: the Helsinki-Uusimaa Hospital District registry of ST-Elevation Acute Myocardial Infarction (HUS-STEMI). European heart journal Acute cardiovascular care 2013;**2**(4):371-8 doi: 10.1177/2048872613501985[published Online First: Epub Date]|.
6. Taylor J. 2012 ESC Guidelines on acute myocardial infarction (STEMI). European heart journal 2012;**33**(20):2501-2 doi: 10.1093/eurheartj/ehs213[published Online First: Epub Date]|.
7. Keeley EC, Boura JA, Grines CL. Primary angioplasty versus intravenous thrombolytic therapy for acute myocardial infarction: a quantitative review of 23 randomised trials. Lancet 2003;**361**(9351):13-20 doi: 10.1016/S0140-6736(03)12113-7[published Online First: Epub Date]|.
8. Boersma E, Primary Coronary Angioplasty vs. Thrombolysis G. Does time matter? A pooled analysis of randomized clinical trials comparing primary percutaneous coronary intervention and in-hospital fibrinolysis in acute myocardial infarction patients. European heart journal 2006;**27**(7):779-88 doi: 10.1093/eurheartj/ehi810[published Online First: Epub Date]|.
9. Rathore SS, Curtis JP, Chen J, et al. Association of door-to-balloon time and mortality in patients admitted to hospital with ST elevation myocardial infarction: national cohort study. Bmj 2009;**338**:b1807 doi: 10.1136/bmj.b1807[published Online First: Epub Date]|.
10. Kushner FG, Hand M, Smith SC, Jr., et al. 2009 focused updates: ACC/AHA guidelines for the management of patients with ST-elevation myocardial infarction (updating the 2004 guideline and 2007 focused update) and ACC/AHA/SCAI guidelines on percutaneous coronary intervention (updating the 2005 guideline and 2007 focused update) a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Journal of the American College of Cardiology 2009;**54**(23):2205-41 doi: 10.1016/j.jacc.2009.10.015[published Online First: Epub Date]|.
11. Bradley EH, Nallamothu BK, Herrin J, et al. National efforts to improve door-to-balloon time results

from the Door-to-Balloon Alliance. Journal of the American College of Cardiology 2009;**54**(25):2423-9 doi: 10.1016/j.jacc.2009.11.003[published Online First: Epub Date]|.

12. Xavier D, Pais P, Devereaux PJ, et al. Treatment and outcomes of acute coronary syndromes in India (CREATE): a prospective analysis of registry data. Lancet 2008;**371**(9622):1435-42 doi: 10.1016/S0140-6736(08)60623-6[published Online First: Epub Date]|.
13. Armstrong PW, Gershlick AH, Goldstein P, et al. Fibrinolysis or primary PCI in ST-segment elevation myocardial infarction. The New England journal of medicine 2013;**368**(15):1379-87 doi: 10.1056/NEJMoa1301092[published Online First: Epub Date]|.
14. Assessment of the S, Efficacy of a New Thrombolytic I, Van De Werf F, et al. Single-bolus tenecteplase compared with front-loaded alteplase in acute myocardial infarction: the ASSENT-2 double-blind randomised trial. Lancet 1999;**354**(9180):716-22
15. Keyt BA, Paoni NF, Refino CJ, et al. A faster-acting and more potent form of tissue plasminogen activator. Proceedings of the National Academy of Sciences of the United States of America 1994;**91**(9):3670-4
16. Assessment of the S, Efficacy of a New Thrombolytic Regimen I. Efficacy and safety of tenecteplase in combination with enoxaparin, abciximab, or unfractionated heparin: the ASSENT-3 randomised trial in acute myocardial infarction. Lancet 2001;**358**(9282):605-13 doi: 10.1016/S0140-6736(01)05775-0[published Online First: Epub Date]|.
17. Zhai M, Chen JL, Qiao SB, et al. Efficacy and safety of recombinant human TNK tissue-typeplasminogen activator in patients with acute myocardial infarction. Chinese Journal of New Drugs 2016;**25**(1):82-86
18. Ross AM, Gao R, Coyne KS, et al. A randomized trial confirming the efficacy of reduced dose recombinant tissue plasminogen activator in a Chinese myocardial infarction population and demonstrating superiority to usual dose urokinase: the TUCC trial. American heart journal 2001;**142**(2):244-7 doi: 10.1067/mhj.2001.116963[published Online First: Epub Date]|.
19. Davies CH, Ormerod OJ. Failed coronary thrombolysis. Lancet 1998;**351**(9110):1191-6
20. Chinese Journal of Cardiology Editorial Board. Reference scheme for treatment of fibrinolytic therapy for acute myocardial infarction. Chinese Journal of Cardiology 1996;**24**(5):328-29. [in Chinese]
21. Cannon CP, McCabe CH, Gibson CM, et al. TNK-tissue plasminogen activator in acute myocardial infarction. Results of the Thrombolysis in Myocardial Infarction (TIMI) 10A dose-ranging trial. Circulation 1997;**95**(2):351-6
22. Cannon CP, Gibson CM, McCabe CH, et al. TNK-tissue plasminogen activator compared with front-loaded alteplase in acute myocardial infarction: results of the TIMI 10B trial. Thrombolysis in Myocardial Infarction (TIMI) 10B Investigators. Circulation 1998;**98**(25):2805-14
23. Weaver WD, Cerqueira M, Hallstrom AP, et al. Prehospital-initiated vs hospital-initiated thrombolytic therapy. The Myocardial Infarction Triage and Intervention Trial. Jama 1993;**270**(10):1211-6

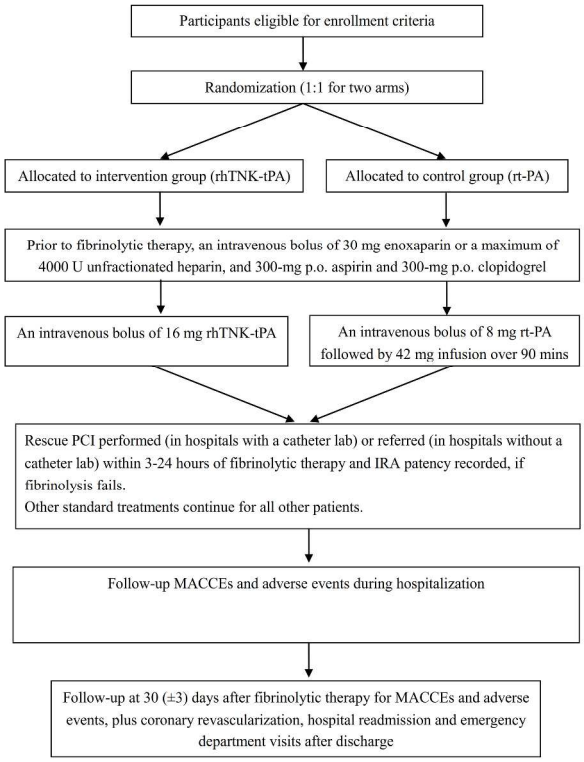

Figure 1

297x420mm (300 x 300 DPI)

	Baseline screening	Monitoring after fibrinolysis	In-hospital follow-up	30-days follow-up
Informed consent	•			
Checking enrollment criteria	•			
Demographical characteristics	•			
Medical history†	•			
Physical examination	•			
Vital signs	•	•	•	
Clinical symptoms	•	•	•	
Cardiac function§	•			
Laboratory examination	•¶	•ξ	•ζ	
Electrocardiogram Ψ	•	•	•	
CK-MB‡	•	•	•	
Randomization	•			
Drug administration	•			
IRA patency, heart failure and cardiac shock		•	•	
Coronary angiography and ultrasonic cardiogram		•	•	
Coronary revascularization			•	•
Concomitant medication	•	•	•	•
MACCEs£		•	•	•
Medical direct expense			•	
Diagnosis and outcome of hospital discharge			•	
Adverse events	•	•	•	•
Health care⊥				•

Figure 2

297x420mm (300 x 300 DPI)

SPIRIT Checking list

Section/Item	Item Number	Description	Checking results	
Administrative information			**Yes /No**	
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	Yes	Recombinant human TNK tissue-type plasminogen activator (rhTNK-tPA) versus alteplase (rt-PA) as fibrinolytic therapy for acute ST-segment elevation myocardial infarction (China TNK STEMI): Protocol for a randomized, controlled, non-inferiority trial
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry.	Yes	The study is registered to Clinical Trials.gov (NCT02835534) with the registry name "The Efficacy and Safety of rhTNK-tPA in Comparison With Alteplase(Rt-PA) as Fibrinolytic Therapy of Acute STEMI".
	2b	All items from the World Health Organization Trial Registration Data Set (**Appendix Table**, available at www.annals.org)	Yes	All items listed in Clinical Trials.gov
Protocol version	3	Date and version identifier	Yes	Version 2.1, 2016-03-03.
Funding	4	Sources and types of financial, material, and other support	Yes	This study is supported by Guangzhou Recomgen Biotech Co., Ltd.
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	Yes	1. Shu-Bin Qiao, Department of Cardiology, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Science and Peking Union Medical College, Principal investigator 2. Hai-Bo Wang, Ping Ji, Xiao-Yan Yan, Chen Yao, Yang-feng Wu,

				Peking University Clinical Research Institute, research design and technical supporting 3. Xing-Shan Zhao, Department of Cardiology, Beijing Jishuitan Hospital, The Fourth Clinical Medical College of Peking University, clinical investigator. 4. Haiyan Xu, Run-Lin Gao. Department of Cardiology, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Science and Peking Union Medical College, clinical investigator. 5. Qin Yang, Guangzhou Recomgen Biotech Co., Ltd., research design.
	5b	Name and contact information for the trial sponsor	Yes	Guangzhou Recomgen Biotech Co., Ltd. Jian-Wen Shi, Tel: 8620-82209996 E-mail: shijianwen@recomgenbio.com
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	Yes	Qin Yang, as the employee of Guangzhou Recomgen Biotech Co., Ltd., has participated in the development of the protocol. But she doesn't engage in data collection, study management, data analysis or data interpretation without decision authority on paper publication.
	5d	Composition, roles, and responsibilities of the coordinating center, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see item 21a for DMC)	Yes	Participants are recruited continuously by physicians who are responsible for fibrinolytic therapy in approximately 150 hospitals across the country. Safety data, especially major bleeding events in the two groups will be reported monthly to the data and safety monitoring committee (DSMB). Through the trial period, DSMB will independently

				evaluate and review the safety data collected and unexpected events according to the rules and guideline of DSMB. For protecting the safety of participants, DSMB will recommend whether the trial should be continued as planned or not after the analysis of these safety data. The primary outcome (major adverse cardiovascular events, MACCEs) and safety indicators will be evaluated by an independent Clinical Endpoint Committee (CEC) which is blinded to treatment assignment. Peking University Clinical Research Institute provides technical supporting for the study, including study design, data management, participants randomization and statistical analysis. Data management department of Peking University Clinical Research Institute is responsible for data quality. Data management related SOPs have been established, including "data management plans" and "data checking plan". Electronic data management system is used in the study and special staff is responsible for data checking. Byrenzhi Co., Ltd. which is independent of the trial administration office is responsible for auditing trial conduct. It is scheduled to audit in the first month after program initiation and one time every three months later.
Introduction				
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining	Yes	Rapid infusion of the recombinant human tissue-type plasminogen activator (rt-PA), in combination with aspirin and heparin, is the most stable and credible strategy for fibrinolytic therapy among

		benefits and harms for each intervention		STEMI patients. However, rt-PA has relatively quick plasma clearance and short half-life, therefore, high dose intravenous infusion is required to maintain effective drug concentration resulting in inconvenience in first-aid. Recombinant human TNK tissue-type plasminogen activator (rhTNK-tPA, Recomlyse®, Guangzhou Recomgen Biotech Co., Ltd.) is a genetically engineered variant of rt-PA, has significant advantages compared with rt-PA, including ease administration, longer half-life and better fibrin specificity.
	6b	Explanation for choice of comparators	Yes	rt-PA has relatively quick plasma clearance and short half-life, therefore, high dose intravenous infusion is required to maintain effective drug concentration resulting in inconvenience in first-aid. Tenecteplase (TNKaseTM, Genentech Inc.), also recombinant TNK-tPA, has been approved by the US Food and Drug Administration (FDA) in 2000, but not entered in Chinese market.
Objectives	7	Specific objectives or hypotheses	Yes	To further validate the efficacy and safety of rhTNK-tPA in Chinese STEMI patients.
Trial design	8	Description of trial design, including type of trial (e.g., parallel group, crossover, factorial, single group), allocation ratio, and framework (e.g., superiority, equivalence, noninferiority, exploratory)	Yes	A multicenter, randomized, open-label, parallel-group, active controlled non-inferiority trial with balanced randomization (1:1).
Methods				
Participants, interventions, and outcomes				
Study setting	9	Description of study settings (e.g., community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study	Yes	The study will be conducted in approximately 150 hospitals where percutaneous coronary intervention is not available across China.

		sites can be obtained		
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centers and individuals who will perform the interventions (e.g., surgeons, psychotherapists)	Yes	Inclusion criteria: (1) aged 18 – 70 years; (2) being diagnosed as acute STEMI, presenting with typical ischemic chest pain lasting for ⩾30 mins and ⩾0.1 mV ST-segment elevation in ⩾2 limb leads or ⩾0.2 mV ST-segment elevation in ⩾2 contiguous precordial leads; (3) duration from onset of symptoms (typical ischemic chest pain) to randomization ⩽6 hours; (4) unable to undergo primary PCI within 1 hr or expected door-to-balloon time >90 mins; (5) willing to participate and provide written informed consent. Exclusion criteria: (1) being diagnosed as any of the following conditions: non-ST-segment-elevation acute MI or unstable angina pectoris; reinfarction; cardiogenic shock; suspected aortic dissection; new-onset left bundle branch block diagnosed by electrocardiogram; (2) having any contradiction to fibrinolysis (referring to Chinese Guideline for Diagnosis and Treatment of STEMI , 2015 Edition), including: systolic blood pressure >180 mm Hg or diastolic blood pressure >110 mm Hg or both, and unresponsive to blood-pressure-lowering treatment; history of intracerebral hemorrhage, cryptogenic stroke, or cerebral ischemic stroke within 3 months; abnormality in cerebral vascular structure or intracranial malignant tumors; active hemorrhage, high risk of hemorrhage, or active peptic ulcer disease; severe facial or head trauma within 3 months; major surgery of head and spinal within 2 months; visceral hemorrhage in the previous 4 weeks; major surgery or trauma in the previous 3 weeks; cardiopulmonary resuscitation lasted >10 mins or endotracheal intubation; vascular punctures with

				hemostasis site unable to be compressed within 2 weeks; current therapy with warfarin, dabigatran, rivaroxaban or glycoprotein IIb/IIIa inhibitors; (3) having other major illnesses that would expose the subject at inordinate risk: indications of cardiac rupture; acute pericarditis, infective endocarditis, acute myocarditis, septic thrombophlebitis or severe infection accompanied with arteriovenous fistula; highly suspected thrombus in left heart chamber, such as mitral stenosis with atrial fibrillation; damage to the central nervous system, such as intracranial tumor, aneurysm, intracranial or spinal canal surgery; severe renal or hepatic dysfunction, or severe hematonosis; maligancy; diabetic hemorrhagic retinopathy or other hemorrhagic ophthalmopathy; history of PCI or coronary artery bypass graft (CABG); fibrinolytic therapy prior to admission; weight less than 50 kg; falling off or other trauma occurring after the onset of ongoing MI; (4) currently participating in other interventional trial; (5) allergic to rhTNK-tPA and/or rt-PA; (6) pregnancy or lactation; (7) inability or unwilling to follow the protocol due to mental disorder; and (8) any other conditions that the investigator judges make the potential participants unfit for participating in the study.
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	Yes	All participants assigned to intervention group will receive an intravenous bolus of 16 mg rhTNK-tPA. The participants assigned to control group will receive an intravenous bolus of 8 mg tPA followed by 42 mg infusion over 90 mins referring to dosing regimen in the TPA/Urokinase Comparisons in China (TUCC) trial.
	11b	Criteria for discontinuing or modifying allocated	Yes	In addition to specified therapies in our study, other medications can

		interventions for a given trial participant (e.g., drug dose change in response to harms, participant request, or improving/worsening disease)		also be administered at the discretion of responsible physicians. The decision to proceed further with PCI after the fibrinolytic therapy is left to the investigator's judgement. Rescue PCI will be performed as soon as possible if fibrinolysis fails. Coronary angiography is suggested to be performed 3 to 24 hours after fibrinolytic therapy. The administration dose of unfractionated heparin could be adjusted to reach an activated clotting time of 250-300 sec during implementing PCI in the catheterization laboratory. Additional dosage of anticoagulant therapy is not necessary for rescue PCI if enoxaparin is used as the primary drug for concomitant therapy. However, if PCI is performed within 3 to 24 hours of receiving fibrinolytic therapy, additional dosage of enoxaparin is also not necessary when last subcutaneous dose is given in 8 hours, and an intravenous bolus of 0.3mg/kg enoxaparin will be added when last subcutaneous dose is given in 8 to 12 hours. In hospitals unable to implement coronary revascularization, participants can be transferred to a collaborative tertiary hospital equipped with facilities to perform PCI or coronary angiography when appropriate.
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (e.g., drug tablet return, laboratory tests)	No	
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Yes	All patients will receive concomitant antiplatelet and anticoagulant therapy prior to fibrinolytic therapy according to guideline-based clinical practice. Enoxaparin is the primary drug for anticoagulant co-therapy, however, unfractionated heparin could be used in replace of enoxaparin if enoxaparin is not available. Antiplatelet therapy

				consists of aspirin in a 300-mg p.o. loading dose prior to fibrinolytic therapy followed by 100 mg daily and clopidogrel in a 300-mg p.o. loading dose prior to fibrinolytic therapy followed by 75 mg daily.
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (e.g., systolic blood pressure), analysis metric (e.g., change from baseline, final value, time to event), method of aggregation (e.g., median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	Yes	The primary study endpoint is the occurrence of MACCEs within 30 days of fibrinolytic therapy, including all causes of death, non-fatal reinfarction, non-fatal stroke (both ischemic and hemorrhagic), PCI due to thrombolysis failure and PCI due to reocclusion. MACCEs will be adjudicated by an independent CEC, the members are not aware of treatment assignment. Secondary endpoints are a) TIMI flow grade 3 in the IRA within 24 hours of fibrinolytic therapy according to coronary angiography (be restricted to participants with available coronary angiography within 24 hours of fibrinolytic therapy). b) IRA patency within 24 hours of fibrinolytic therapy diagnosed by non-invasive clinical indexes, IRA patency can be determined if any two out of the following 4 items (3+4 excluded) can be achieved: 1) reduction of elevated ST-segment ⩾50% by electrocardiogram is achieved within 60-90 mins of receiving fibrinolytic therapy; 2) the time to peak cTn concentration is advanced to ⩽12 hours of symptom onset and the time to CK-MB concentration is advanced to ⩽14 hours of symptom onset; 3) significant relief of chest pain within 2 hours of fibrinolytic therapy; 4) presence of reperfusion arrhythmia within 2 to 3 hours of fibrinolytic therapy, including accelerated idioventricular rhythm, sudden improvement or disappearance of atrioventricular block or bundle branch block, and transient sinus bradycardia or sino-auricular block with or without hypotension

				among patients with inferior wall MI. c) The occurrence of MACCEs during hospitalization. d) All-cause mortality during hospitalization and within 30 days of fibrinolytic therapy. e) Cardiovascular mortality during hospitalization and within 30 days of fibrinolytic therapy. f) The occurrence of reinfarction during hospitalization. g) The occurrence of new onset or worsen heart failure during hospitalization. h) The occurrence of cardiac shock during hospitalization. i) The occurrence of coronary revascularization within 30 days of fibrinolytic therapy.
Participant timeline	13	Time schedule of enrollment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (Figure).	Yes	The study consists of three phases: baseline assessment, in-hospital follow-up and follow-up after discharge. For each phase the main information collected are described below. Figure 2 shows an overview of the most important data.
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	Yes	Data from our unpublished CPACS-3 study in China showed that the incidence of MACCEs was 17.2% among STEMI patients within 30 days of fibrinolytic therapy. We, therefore, assumed that the incidence of MACCEs would be 17% in the control group in this study and we further set a 3% of absolute rate increase as the non-inferiority margin, corresponding to a non-inferiority relative risk margin of 1.176. With these assumptions, 2,923 participants in each arm of the study are required to provide 80% power with the use of two-sided significance level of 5%. Assuming a 5% loss to follow-up rate, enrolment of 6,200 patients (or 3,100 participants per arm) is projected to yield the necessary number of events.
Recruitment	15	Strategies for achieving adequate participant enrollment to reach target sample size	Yes	The physicians of participating hospitals explain the purpose of the study, the procedures and risks/benefits of participation to potential

				subjects or their relatives. After consultation with potential participants and screening their clinical information, the physicians inform patients whether they are eligible for the study.
Assignment of interventions (for controlled trials)				
Allocation Sequence generation	16a	Method of generating the allocation sequence (e.g., computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (e.g., blocking) should be provided in a separate document that is unavailable to those who enroll participants or assign interventions.	Yes	Central randomization via interactive web response system (IWRS, Medidata Balance) will be carried out by Peking University Clinical Research Institute, which is independent to the trial administration office. Dynamic randomization will be conducted with varying block size and will be stratified for research center, age (≤60 years vs. >60 years) and the time from symptom onset to randomization (≤3 hours vs. 3~6 hours). The participant is randomized to either intervention group (rhTNK-tPA) or control group (rt-PA) on 1:1 ratio.
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (e.g., central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	Yes	The allocation sequence is computer-generated and the randomization list is not known to the investigators.
Implementation	16c	Who will generate the allocation sequence, who will enroll participants, and who will assign participants to interventions	Yes	Central randomization is carried out by Peking University Clinical Research Institute, which is independent of the trial administration office. Clinical investigators enroll participants and assign participants to interventions according to randomization.
Blinding (masking)	17a	Who will be blinded after assignment to interventions (e.g., trial participants, care providers, outcome assessors, data analysts), and how	No	Given the nature of intervention, allocation status cannot be blinded to the participants and investigators due to different methods of administration for intervention and control treatments.
	17b	If blinded, circumstances under which unblinding is	No	

		permissible, and procedure for revealing a participant's allocated intervention during the trial		
Data collection, management, and analysis				
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (e.g., duplicate measurements, training of assessors) and a description of study instruments (e.g., questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol.	Yes	Described in the Figure 1 and Figure 2. The study consists of three phases: baseline assessment, in-hospital follow-up and follow-up after discharge. For each phase the main information collected are described below. Figure 2 shows an overview of the most important data. Before the patient's discharge, the responsible investigator needs to collect all data in relevance to identify and diagnose the study outcomes, using a uniform e-CRF. All randomized participants are planned to be followed-up at 30 (±3) days after fibrinolysis, with both face-to-face interview and telephone follow-up acceptable. Please refer to the following section of primary and secondary outcomes for details.
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	Yes	Colleting contact information and providing free treatment to maintain follow-up adherence.
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (e.g., double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol.	Yes	Data management department of Peking University Clinical Research Institute is responsible for data quality. Data management related SOPs have been established, including "data management plans" and "data checking plan". Electronic data management system is used in the study and special staff is responsible for data checking.
Statistical	20a	Statistical methods for analyzing primary and	Yes	Based on ITT principle, full analysis set (FAS) consisting of all

methods		secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol.		randomized patients is used. The occurrence of MACCEs within 30 days of fibrinolytic therapy is compared with Chi-squared test or Fisher's exact as required, and multivariate logistic regression model is used to adjust for treatment effect on baseline characteristics if there is statistical significant difference between two groups. The secondary endpoints will be analyzed with the chi-square test or Fisher's exact test as appropriate to compare the difference between two groups. Adverse events in the two arms, including bleeding events, will also be compared using the chi-square test or Fisher's exact test as appropriate.
	20b	Methods for any additional analyses (e.g., subgroup and adjusted analyses)	Yes	If inequality of baseline characteristics is detected, the confounding factors will be defined and multivariate logistic regression model will be used to adjust for covariate effects.
	20c	Definition of analysis population relating to protocol nonadherence (e.g., as-randomized analysis), and any statistical methods to handle missing data (e.g., multiple imputation)	Yes	Both intention-to-treat (ITT) and per-protocol analyses will be done for the primary analyses as is recommended for non-inferiority studies, but principally with reference to per-protocol analysis.
Monitoring				
Data monitoring	21a	Composition of DMC; summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed.	Yes	The data and safety monitoring committee (DSMB) is composed of two cardiovascular physicians and one neurological expert who are recommended and appointed by Peking University Clinical Research Institute. Safety data, especially major bleeding events in the two groups will be reported monthly to DSMB. Through the trial period, DSMB will independently evaluate and review the safety data collected and unexpected events according to the rules and guideline of DSMB.

				The primary objective of the DSMB is to monitor the safety of the interventions and the validity and integrity of the data from the study. Additionally, the DSMB will evaluate the pace of recruitment and will make recommendations regarding the continuation, modification, or termination of the study. The following will ensure the independence of the DSMB: •Members of the DSMB will not participate as investigators in any study under review and will not be supervised by study investigators. •Members of the DSMB must not have a direct interest in knowing or influencing trial outcome or have a financial or intellectual interest in the outcome of any studies under review. •DSMB members must disclose all pharmaceutical companies, biotechnology companies, and CROs in which they hold financial interest. Members must disclose all consultancies (direct or indirect) with pharmaceutical companies, biotechnology companies, and CROs.
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial		As this trial is a non-inferiority trial, we do not consider an interim analysis appropriate. As an interim analysis will not be performed, statistical early stopping criteria will not be applied. However, DSMB has full access to the safety data and will do a thorough review of these after 10%, 25% and 50% patients completing the study procedure, and may recommend stopping the study anytime based on safety concerns.
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial	Yes	Safety data, especially major bleeding events in the two groups will be reported monthly to the data and safety monitoring committee (DSMB). Through the trial period, DSMB will independently

		interventions or trial conduct		evaluate and review the safety data collected and unexpected events according to the rules and guideline of DSMB. For protecting the safety of participants, DSMB will recommend whether the trial should be continued as planned or not after the analysis of these safety data.
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Yes	Byrenzhi Co., Ltd. which is independent of the trial administration office is responsible for auditing trial conduct. It is scheduled to audit in the first month after program initiation and one time every three months later. The auditing contents include complete status and upgrading of study files, signature of informed consent, source data verification, protocol violation and serious adverse events. A written inspection report will be drafted after audition and the report will be sent to principle investigator and sponsor.
Ethics and dissemination				
Research ethics approval	24	Plans for seeking REC/IRB approval	Yes	The protocol and informed consent form should be reviewed and approved by the Institutional Review Boards of all participating hospitals.
Protocol amendments	25	Plans for communicating important protocol modifications (e.g., changes to eligibility criteria, outcomes, analyses) to relevant parties (e.g., investigators, RECs/IRBs, trial participants, trial registries, journals, regulators)	Yes	Protocol revision should get the approval of both principle investigator and Peking University Clinical Research Institute. In addition, any protocol modifications should be reported to other investigators, RECs/IRBs and trial participants. And the registry information will be revised in trial registries.
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorized surrogates, and how (see item 32)	Yes	Informed consent was done by physicians in the participating hospitals.

	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	No	
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	Yes	The files including personal information will be locked in a cabinet. Only special study staffs, auditor and IRBs have limited access to these files for study purpose. The CRF for collecting research information does not include personal information. And the data published will not include any personal information.
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	Yes	Principal investigators acted as clinical investigators in this clinical study, but received no direct payment from Guangzhou Recomgen Biotech Co., Ltd for their roles in conducting the study.
Access to data	29	Statement of who will have access to the final trial data set, and disclosure of contractual agreements that limit such access for investigators	Yes	Principal investigators have full access to the final trial data set.
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	No	The treatments in intervention and control group are regular in clinical setting without excess harms to participants. The safety advents will be disposed as regular clinical side effects.
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, health care professionals, the public, and other relevant groups (e.g., via publication, reporting in results databases, or other data-sharing arrangements), including any publication restrictions	Yes	The study results will be published in medical journal or communicated with health care professionals in academic conference, but all the personal information of participants is prohibited to disclose.
	31b	Authorship eligibility guidelines and any intended use of professional writers	Yes	Research related investigators are eligible for the authorship.

	31c	Plans, if any, for granting public access to the full protocol, participant-level data set, and statistical code	Yes	Full protocol will be published in medical journal and everybody has public access for it. Only principle investigator has full access to participant-level data set, and statistical code. Other staffs can get the access to study data only if they get grant from principle investigator.
Appendices				
Informed consent materials	32	Model consent form and other related documentation given to participants and authorized surrogates	Yes	Informed consent has been designed, and both participants and investigators should sign inform consent before participating in the study.
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	No	Biological specimens will not be stored for future study.